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Comparing growth trajectories with scale analysis

Masteroppgave i Ocean Resources

Veileder: Ivain Martinossi

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Norges teknisk-naturvitenskapelige universitet  
Fakultet for naturvitenskap  
Institutt for biologi



Kunnskap for en bedre verden



## Sammendrag

På tvers av varierende breddegrader, presenteres et bredt spekter av forskjellige klima store evolusjonære utfordringer, som presser fisk til bemerkelsesverdige tilpasninger som en respons. Mange av disse tilpasningene er fortsatt ukjente, og det er viktig å øke kunnskapen vår om dem, spesielt med tanke på den dokumenterte klimaendringen som har blitt registrert i de siste tiårene. For å håndtere dette, undersøkte vi effekten av breddegrad på livshistoriestrategiene til Tangkutling (*Pomatoschistus flavescens*) ved å sammenligne vekstmønster hos populasjoner fra ulike breddegrader langs den norske og svenske kysten. Vi analyserte skleritt-formasjonen i skjell for å finne informasjon om fiskens vekst gjennom livet, med fokus på å forstå hvordan fisk tilpasser seg varierende miljøforhold langs en breddegradsgradient.

Skjellanalysene viste at fisken i sør hadde en lengre vekstsesong, men at fisken i nord likevel kunne vokse mer. I tillegg viste fisk fra nord en høyere veksthastighet i første fase av livet, til tross for kortere vekstsesonger og kaldere klima. Dette kan være forårsaket av en mot-gradient tilpasning, der arten viser økt vekst tross mindre gunstige forhold.

Flere av resultatene antyder at i nordlige populasjoner kan en betydelig del av populasjonen reproducere i sitt andre leveår, selv om arten generelt er kjent å reproducere innen et års tid.

Funnene våre fremhever de betydelige effekten av breddegrad på vekst og livshistoriestrategier til Tangkutling, ved å demonstrere at fisken går fra å ha en hovedsakelig årlig syklus til en hovedsakelig toårig syklus i nord på grunn av miljøforhold. Dette er en fullstendig endring av reprodutiv strategi. Fremtidig forskning bør utforske avls-strategier blant 2 år gamle fisk, samt forskjeller i vekst hos individer fra de forskjellige populasjonene for å tilby dypere innsikt i de tilpasningsmekanismene til denne arten.

## Abstract

Across varying latitudes, a wide range of different climates presents great evolutionary challenges, pushing fish to remarkable adaptations as a response. Many of these adaptations are still unknown, and it is important increase our knowledge about them, especially with the changing climate that has been documented in recent decades. To tackle this, we investigated the impact of latitude on the life history strategies of two-spotted gobies (*Pomatoschistus flavescens*) by comparing growth trajectories of populations from different latitudes along the Norwegian and Swedish coast. We analyzed sclerite formation in scales to extract information on growth throughout the life of individual fish, with an emphasis on understanding how fish adapt to varying environmental conditions along a latitudinal gradient.

Scale analysis showed that the fish in the South experienced a longer growing season, but that the fish in the North still were able to grow more. Additionally, we found that northern fish exhibited a higher initial growth rate, despite shorter growing seasons and colder climates. This could correspond to counter gradient adaptation, where species show accelerated growth despite less favorable conditions.

Several lines of evidence suggest that in northern populations a substantial part of the population can reproduce in its second year of life, although the species is generally thought to be annual.

Our findings highlight the significant latitudinal effects on the growth and life history strategies of two-spotted gobies, by demonstrating that the fish transition from a mostly annual cycle to a mostly biannual cycle in the North due to environmental conditions. This is a complete change of reproductive strategy. Future research should explore the breeding strategies among 2 years old fish and differences in growth for individuals from the populations, offering deeper insights into the adaptive mechanisms employed by this species.

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# 1 Introduction

Understanding how fish adapt to a changing climate is becoming increasingly important. The increasing concentration of greenhouse gases in the atmosphere has triggered a rise in temperatures, and with the ocean acting as the primary repository of heat for the planet, there has been an accelerating change in sea temperatures over recent decades (Reid et al., 2009, Garcia-Soto et al., 2021). In recent years it has been documented cases of fish changing their timing of reproduction, age of maturity, growth and survival, due to climate change (Crozier and Hutchings, 2014). It is therefore important to understand better how fish life history strategies change with climatic conditions. This can be done using natural gradients of climate for species spawning large latitudinal ranges. In this study we focus on the two-spotted goby (*Pomatoschistus flavescens*), looking at populations located at various latitudes along the Norwegian and Swedish coasts. By examining these populations, we aimed to determine the impact of latitude on their life history strategies. The differing climates across these latitudes can significantly influence the fish, and studying this latitudinal variation could provide valuable insights into how environmental changes might affect them in the future.

The life history of fish decides the pattern of how they distribute their resources for growth, survival, and reproduction during their lifespan (Williams, 1966, Teichert et al., 2017). Local environmental conditions play a great role in shaping a range of successful strategies (Teichert et al., 2017). One major local conditions that fish life history strategies must adapt to is water temperature, and it is an important factor for the timing of reproduction is because temperature majorly decides the rate of metabolism in ectotherms. This means that an optimal temperature can decrease the time it takes to reach sexual maturity and for juveniles to grow (Burel et al., 1996). Temperature is greatly influenced by seasonality. (Varpe, 2017). Defined as the occurrence of biotic and abiotic events within specific periods of the year (Lieth, 2013), seasonality profoundly impacts the timing of annual activities and developmental stages for most species. A key adaptation to seasonality involves optimizing the timing of reproduction to preserve the best possible reproductive value (Varpe, 2017). As seasonality changes with latitude, characterized by longer winters and lower temperatures in the north, it will greatly affect fishes life history strategies as temperature is one of the key factors for choosing when to reproduce (Conover, 1992). Within some fish species, the spawning always occurs around the same temperature regardless of latitude, leading to later starts and earlier finishes at higher latitudes, consequently affording offspring from lower latitudes more time for growth (Conover, 1990). Temperature is considered as one of the most important environmental factors for determination of growth of fish in early life stages, with optimal temperatures enhance growth rates, potentially resulting in larger offspring from lower latitudes (Shoji et al., 2011, Conover, 1990).

Fish are ectotherms, which means that small temperature changes can have a great impact on several fundamental processes, such as behavior, reproduction, metabolism, and growth (Albouy et al., 2023, Lopes et al., 2022). Changes in temperature linked with seasonality will therefore affect all these processes. Temperature plays a big role in regulating the consumption rate, leading many species to endure the colder periods with reduced or

suspended feeding. This will eventually result in the metabolic cost exceeding the energy intake, causing an energy deficit and a negative scope for growth (Hurst, 2007). While some species have the capacity to grow during winter, others stop their growth completely. These species will also most likely experience slower growth in the periods close to winter (late fall and early spring) (Hurst, 2007, Fukuwaka, 1998). It has been indicated that the threat of winter mortality plays a dominant role in the evolution of growth rates. For many ectotherms, the size of offspring and winter survival have been shown to have a strong correlation, with larger offspring having a lower mortality than small offspring. Since the duration of winter increases with latitude, the level of mortality related to size should also increase with latitude (Conover, 1992, Schultz et al., 1996).

The increased chance of winter mortality and unfavorable growing conditions in the higher latitudes has led to many species adapting in different ways to be able to inhabit high latitude environments. Geographical variation in life-history strategies is common among populations occurring at different latitudes and has been observed in several species of marine fish in particular (Schultz et al., 1996). For example, Conover and Present (1990) found that Atlantic silversides (*Menidia menidia*) had the same body size at the end of the first growing season, despite the growing season being a lot shorter in higher latitudes than in lower latitudes. This was because the high-latitude fish grew faster within the growing season and had adapted for a rapid elevation of growth rate during the short time available for high temperatures. This phenomenon is called counter gradient variation in growth rate (Conover and Present, 1990). Temperature adaptation is also a way of adapting to changes in temperature with increasing latitude by having the capacity to shift the temperature threshold for growth (Yamahira and Conover, 2002). This means that higher latitude fish could start growing at lower temperature, in order to extend the growth period. Finally, another way of changing reproductive strategy in response to high latitude is by postponing or skipping reproduction in certain years, which could be beneficial for example when the body condition that is required for breeding are not fulfilled (Varpe, 2017).

Postponement of reproduction will allow fish to reallocate the energy that normally would be used for reproduction towards somatic growth. This results in an overall weight gain and thereby a growth in size (Kissil et al., 2001). Size is known to be strongly correlated with fecundity, so there is reason to believe that some fish can gain better reproductive value by postponing their reproduction to grow. Predation risk can also be size dependent, so that reaching a certain size can increase survival drastically. For example, a study by Berglund and Rosenquist (1986) on prawns showed that the postponement of reproduction decreased the susceptibility to predators in the year they were non-reproducing. All of these examples of adaptation demonstrate how important the understanding of the effect of latitude can be, and that there are several different ways to explore how fish adapt.

A good way to learn about the life history of fish is to read scales. They can give information about the growth trajectory since birth. On the scale surface, there are a series of ridges, which are called sclerites (Fukuwaka, 1998). These ridges grow in the intercellular space, and are formed by two overlapping osteoblasts in the scale (Yamada, 1971). Osteoblasts are cells

that is used for bone formation (Hu et al., 2016). It is demonstrated that fish within the same species and climate generally uses the same time to form a new sclerite (Flem et al., 2017), meaning that the number of sclerite can give a relative age for fish experiencing the same growth conditions. Moreover, the size of scales is generally associated with body size, as the distance between sclerites can be indicative of the amount of growth realized during a specific period (Fukuwaka, 1998, Das, 1994). For example, during periods of slow growth, it is also generally accepted that the spacing between sclerites will decrease (Fukuwaka, 1998).

This project focuses on the two-spotted gobies (*P. flavescens*), with the aim to compare life history strategies at different latitudes. The two-spotted goby is expected to be an annual species where the fish are hatched in spring and live until their second summer, although Johnsen (1945) has previously suggested that they could reach the age of 2 years in northern waters due to a delay in reaching maturity. Even though they usually only live for one year and have one reproductive season, they can spawn several times in the course of that season (Johnsen, 1945). Most of their growth is believed to be completed by the autumn, and that there is little to no growth after November, before it starts again in the spring. The growth is also expected to slow down during spawning due to the energy trade-off between growth and reproduction. After spawning they disappear from the shore, which could be a result of migration to deeper waters or mortality (Collins, 1981). The breeding season for the two-spotted goby that lives along the Scandinavian coast usually lasts between April or May to July, but because of variations of temperatures at different latitudes, the length of the season varies (Skolbekken and Utne-Palm, 2001). Fish at lower latitudes are able to produce more consecutive broods than those at higher latitudes, and this is likely because of the shorter breeding season at higher latitudes (Amundsen, 2018). In 2022, populations north of Trondheim were sampled for the first time, as a part of a latitudinal study ranging from 58.2N to 68.6N. This study revealed that some two spotted gobies from high-latitude populations were unexpectedly large. We suspect that these large individuals are two years old because it is unlikely that fish could grow larger in the North than in the South in a single season with colder water temperatures and a shorter productive season. The goal of this project is therefore to confirm the age of large fish from Northern populations, and more generally investigate the two spotted goby changes in life history strategies with latitudes.

## 2 Method

### 2.1 Sample collection

All fish samples used during this project were collected during the fieldwork for the DYNAMAR project in the spring and summer of 2022 on the Norwegian coast. Fish were sampled from six populations (Figure 1), with 5 replicate sublocations per population. Fish were caught by snorkeling field workers using dip nets and measured for length on land. A subsample of the individuals was collected in tubes with 95% ethanol. 2765 fish were measured to draw size distributions, but only 3 per sex per sublocation were collected. This was done 3 times between late April and mid July, for a total of 180 collected fish.



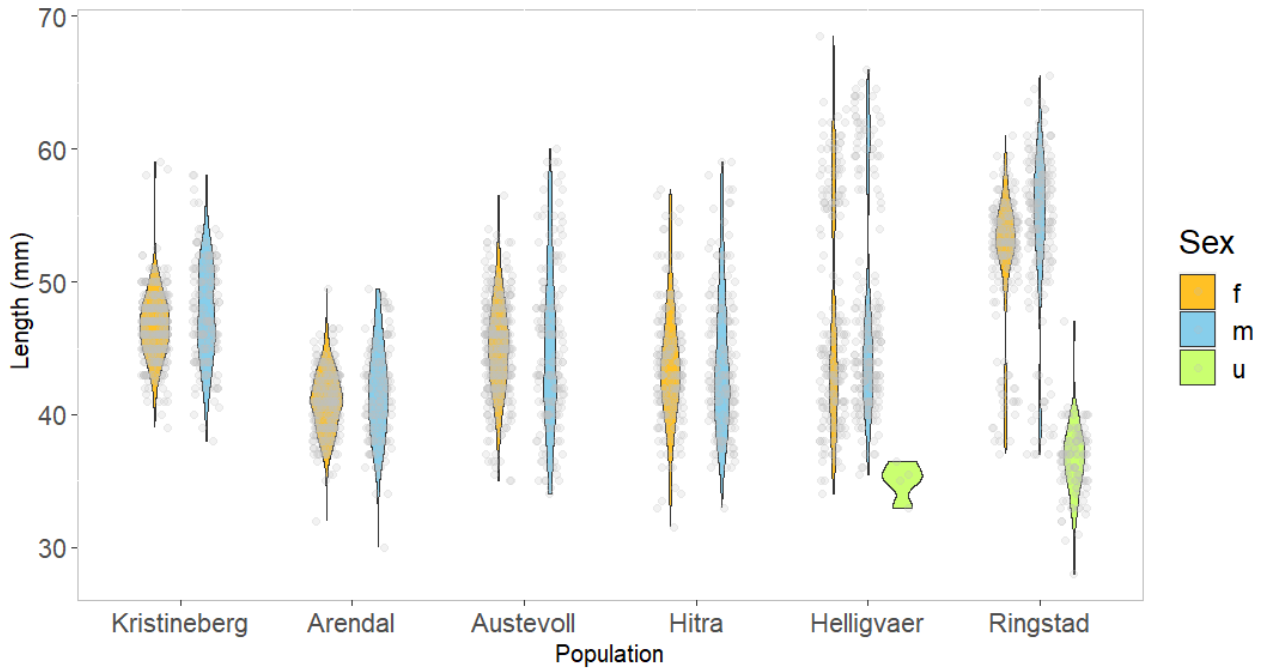
*Figure 1: Map of the 6 populations sampled in the DYNAMAR project. Each arrow indicates one population, with Kristineberg as the most southern location and Ringstad as the most northern location. Map background from Google maps.*

## 2.2 Experimental design

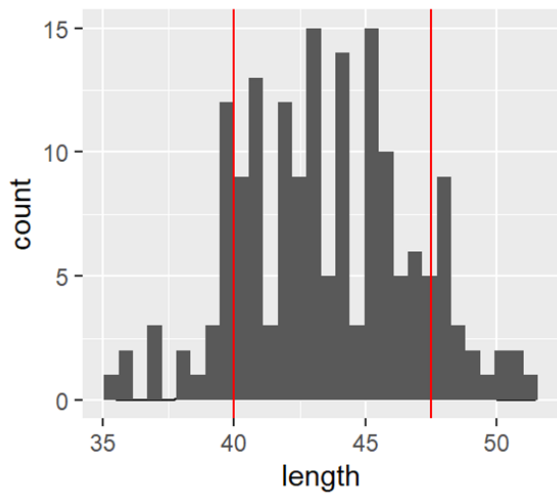
A plot of the size distributions of the fish sampled at the first sampling occurrence was made for each sex and population (Figure 2). These size distributions were either unimodal or bimodal, depending on the populations. The two northernmost populations of Helligvær and Ringstad had clear bimodal distributions for both males and females (Figure 2). Bimodal distributions suggest two age classes, but since the species is believed to be annual, this required further investigation. We defined a priori two classes of fish for each population: Small and Large. For populations with a bimodal size distribution, we considered that fish of the Large class were fish of size within 75 % of the size distribution, around the median of the larger group (Figure 3). Small fish were of size within 75 % of the smaller size distribution around the median of the smaller group. When the size distribution was unimodal, individuals of the Small class were from the main distribution (within 75% of the median), and Large fish were the largest individuals sampled, if available outside of the 75% range. When sampling individuals for scale analysis, we attempted to cover as much as possible of the 75% of each size distribution. We did this by using one fish close to the middle of the distribution and two fishes of sizes close to the edges of 75% of the distribution.

Since the fish had laid in tubes with alcohol for over a year, it was suspected that they may have shrunk in size. 20 fish were therefore measured and compared their total length to the total length that was recorded during the fieldwork on the same live individuals. Fish preserved in ethanol were indeed smaller than when measured in the field, and we fitted a

linear model to this pilot data to correct the size of alcohol-preserved fish. The formula was  $y=1.0399x-0.2369$ , where  $y$  is the size the fish would have had in the field, and  $x$  is the size measured after alcohol preservation.



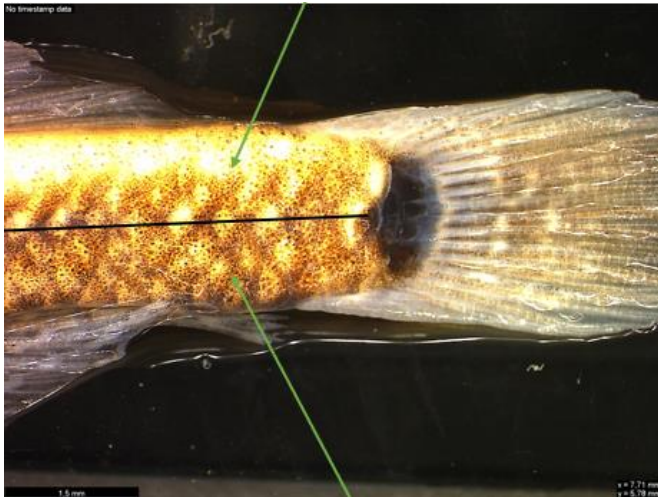
**Figure 2: Size distribution of adult *P. falvenscens* at the first sampling time of the 2022 field season.** Violin plots were used to emphasize if distributions were unimodal or bimodal. Each dot represents one individual measured in the field. *f*=Female, *m*=Male, *u*=Unsexed, Populations are from left to right in order of latitude (South to North).



**Figure 3: How 75% of the distribution was drawn.** This figure shows an example of how 75% of the distribution was drawn to find the samples within the different classes. The red lines represent both ends of 75% of the distribution.

### 2.3 Scale extraction:

Prior to scale extraction in the lab, individual fish were identified with a random number to avoid any biases with respect to the population of origin when analyzing them. Scales were picked with tweezers from 3 places on the fish. The scales were always picked behind the dorsal fin. They were picked on the right and left side above the lateral line and on the right or left side under the lateral line (Figure 4). In some cases, it was hard to find good-quality scales. Usually, 3 scales from the same area were enough to find a good one, but in the cases up to 10 scales were picked to try to find a good one. There were also a few cases where it was not possible to obtain any good-quality scales.



*Figure 3: Scale extraction. The arrows point to the area where the scales were extracted from.*

Under a stereomicroscope, the cuticle that was left on the scales was scraped off with tweezers and a needle on both sides of the scale to make the sclerites visible. It was important that the scales lay in a drop of water so that they did not get dry and shrink.

Photos of the scales are taken with the use of a Leica MZ 95 microscope, Leica MC170 HD camera, and LAS X software.

### 2.4 Analysis of photographs

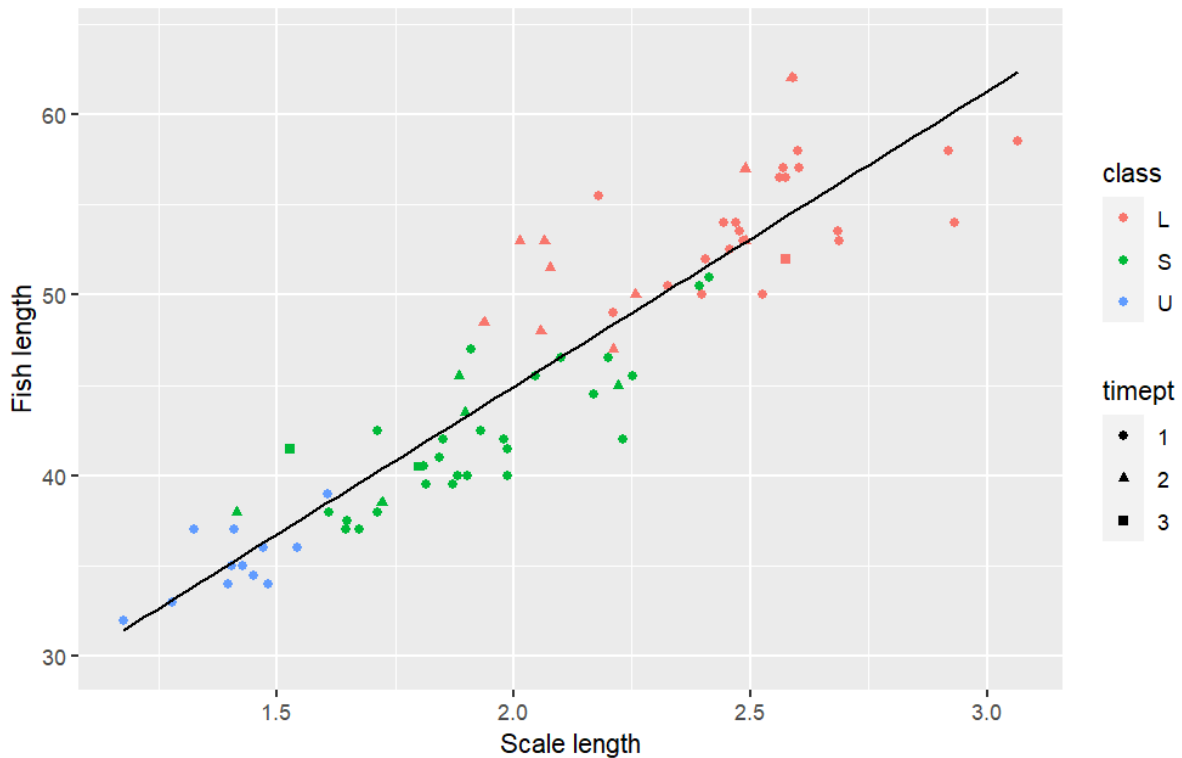
The length between the sclerites was measured in ImageJ. This was done by measuring the length between 3 and 3 consecutive sclerites. The measurements were typed into Excel, and then made into a dataset that was used for further analysis in R. All measurements in ImageJ were calibrated from a picture of a calibration ruler and by setting the same scale for all measurements. All pictures were taken with the same settings on the imaging software and the same level of magnification on the microscope.

### 2.5 Data analysis

All data were analyzed using the software R v.4.2.1 (R Core Team, 2023). The plots were made using the package ggplot2 (Wickham, 2016). Statistical analysis consisted of fitting linear models comparing scale characteristics of the different populations and size classes, and analyzing them with ANOVAs using the package car (Fox and Weisberg, 2019), and post-hoc tests using the postHoc package (Labouriau, 2020).

## 3 Results

### 3.1 Relationship between fish length and scale length

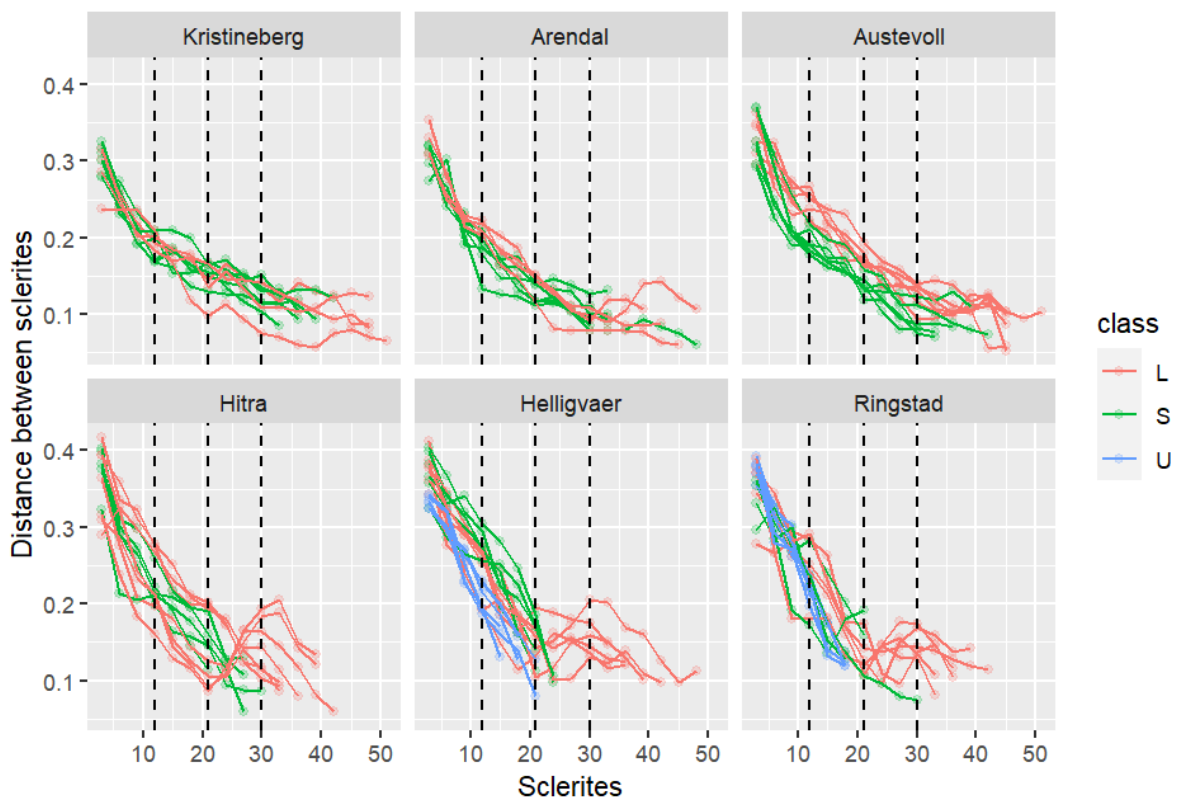


**Figure 5: Relationship between fish length and scale length.** Both axes' units are in mm. Each color represents a size class: L=Large, S=Small, U=Unsexed. Each dot represents one individual fish for which tree scales were sampled. The different dot shapes represent the different time points when the fish were sampled. Circle= time point 1, Triangle= time point 2, Square= time point 3. The black line is the linear regression line. Some fish of the Small class may be larger than Large class fish, due to differences in average size between populations. Within each population, Small class fish are always smaller than Large class fish.

I used the intersclerite distance as a proxy for growth in the two-spotted goby. To support this method, I wanted to see how much of the variation in fish length was explained by the length of the scale. This was done by fitting a linear model with fish length as a response variable and scale length as the explanatory variable. Figure 5 shows the regression line of that model. The elevated R squared value suggested that variation in body length of the fish is largely explained by variation in scale length (R-squared = 0,8261 and a p-value < 2,2e-16). Consequently, intersclerite distance may be a good proxy for growth.



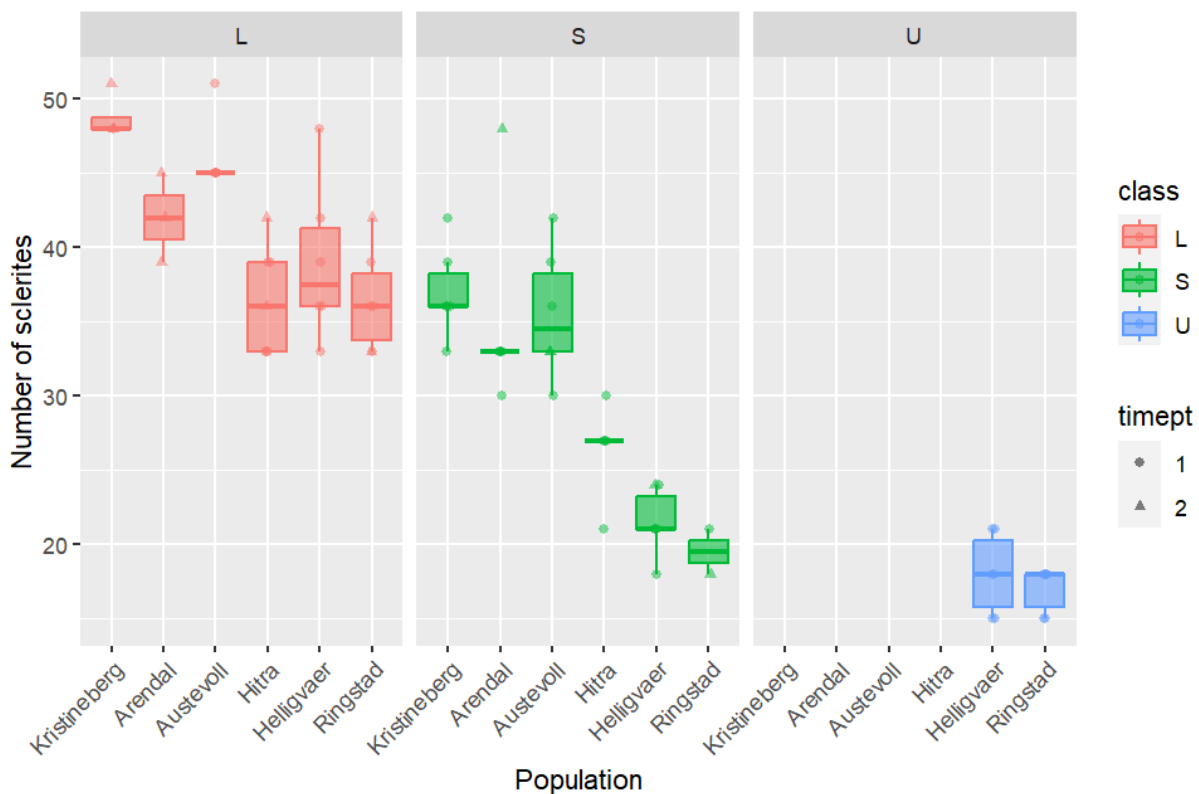
### 3.2 Growth trajectories



**Figure 6: Growth trajectories of fish of the different populations and size classes.** Each panel represents a population, from South to North in order of reading. Each line represents one individual fish where tree scales were sampled and averaged, with the distance between sclerites on the y axis and the sclerite number counted from the focus on the x axis. The distance between the sclerites is measured in mm, and for the space between three consecutive sclerites at a time. The different colors represent the size class of the fish: L=Large, S=Small, U=Unsexed. The dashed lines separate different phases of the growth trajectories that were defined a posteriori to compare growth trajectories.

To be able to see the different growth patterns of the fishes, plots was made with the distance between the sclerite as a function of the sclerite number, where sclerites are counted from the focus, which is the earliest growth (Figure 5). This plot allows to visualize how the different size classes differ in the length of their growth period (total number of sclerites) and in speed of growth over time. In Figure 6, we see that there was a longer growth trajectory for both Small and Large individuals in the South than in the North and we see that the Unsexed individuals only existed in the two northernmost populations and that their trajectory were very steep and short. A steep growth trajectory indicates that growth decreases in speed very quickly. In the next sections, we will analyze the main characteristics of these growth curves with statistical models to compare the different populations and classes of fish.

### 3.3 Number of sclerites



**Figure 7: Average number of sclerites for fish from different populations and size classes.** Populations are ordered from South to North in reading order. Each panel represents a size class: L=Large, S=Small, U=Unsexed. Each dot represents one individual fish where three scales were sampled and averaged. The dot shape represents the different timepoints when the fish were sampled. Circle= time point 1, Triangle= time point 2.

We assume that sclerites on scales deposit regularly over time, and that sclerites do not deposit when growth is stopped during winter. The number of sclerite can therefore tell us something about the age of the fish within a location. Fish from the same location that have more sclerites than others are thereby older than those with less sclerite, because they should experience the same environment and thus the same period of halted growth in winter. When comparing different populations however, the number of sclerites can also tell us something about the environment, with more sclerites meaning that the fish have a longer period for growth available. Thus, we expected that Large class fish would have more sclerite than Small class fish from the same population, if the Large fish was indeed older than the Small fish. We also expected that the fish in the South would have generally more sclerites than those in the North because of the longer winters in the North. Populations and classes differed significantly in the number of sclerites (Table 1), with Large class fish and southern populations having generally more sclerites (Figure 6). A post hoc test indicated that the three southernmost populations and the three northernmost populations formed two distinct groups of sclerite numbers (Table 2). This is likely due to the environmental effect of longer winters in the North. Within each population, Large class fish had more sclerites, indicating older age of the Large fish. In figure we see that for the Northern populations, the median sclerite number almost doubles between Small and Large class fish. There was no significant effect of the interaction of location and class on sclerite number however.

The unsexed individuals were not included in the statistical model for the number of sclerites, since they only existed in two of our populations. However, we did fit a model to the subset of the data with only the two northern populations, where the tree classes were present. Class affected sclerite number in these populations (Table 3), and a post hoc test indicated that the Small and Unsexed individuals were significantly different from the Large individuals in the number of sclerites, but Small and Unsexed did not differ (Table 4).

**Table 1:** Results from ANOVA test for difference in number of sclerites among populations and class.

	<b>Sum sq</b>	<b>Df</b>	<b>F-value</b>	<b>p-value</b>
Population	1824,73	5	21,19	<b>&lt;0,001</b>
Class	2209,87	1	128,31	<b>&lt;0,001</b>
Residuals	964,48	56		

**Table 2:** P-value from post hoc pairwise comparisons in number of sclerites between populations. The populations are put into different groups depending on which population they are significantly similar to.

	<b>Group</b>	<b>Kristineberg</b>	<b>Arendal</b>	<b>Austevoll</b>	<b>Hitra</b>	<b>Helligvær</b>
Kristineberg	B					
Arendal	B	0,48910				
Austevoll	B	0,82866	0,98761			
Hitra	A	<b>&lt;0,001</b>	<b>&lt;0,001</b>	<b>&lt;0,001</b>		
Helligvær	A	<b>&lt;0,001</b>	<b>&lt;0,001</b>	<b>&lt;0,001</b>	0,99184	
Ringstad	A	<b>&lt;0,001</b>	<b>&lt;0,001</b>	<b>&lt;0,001</b>	0,95165	0,99956

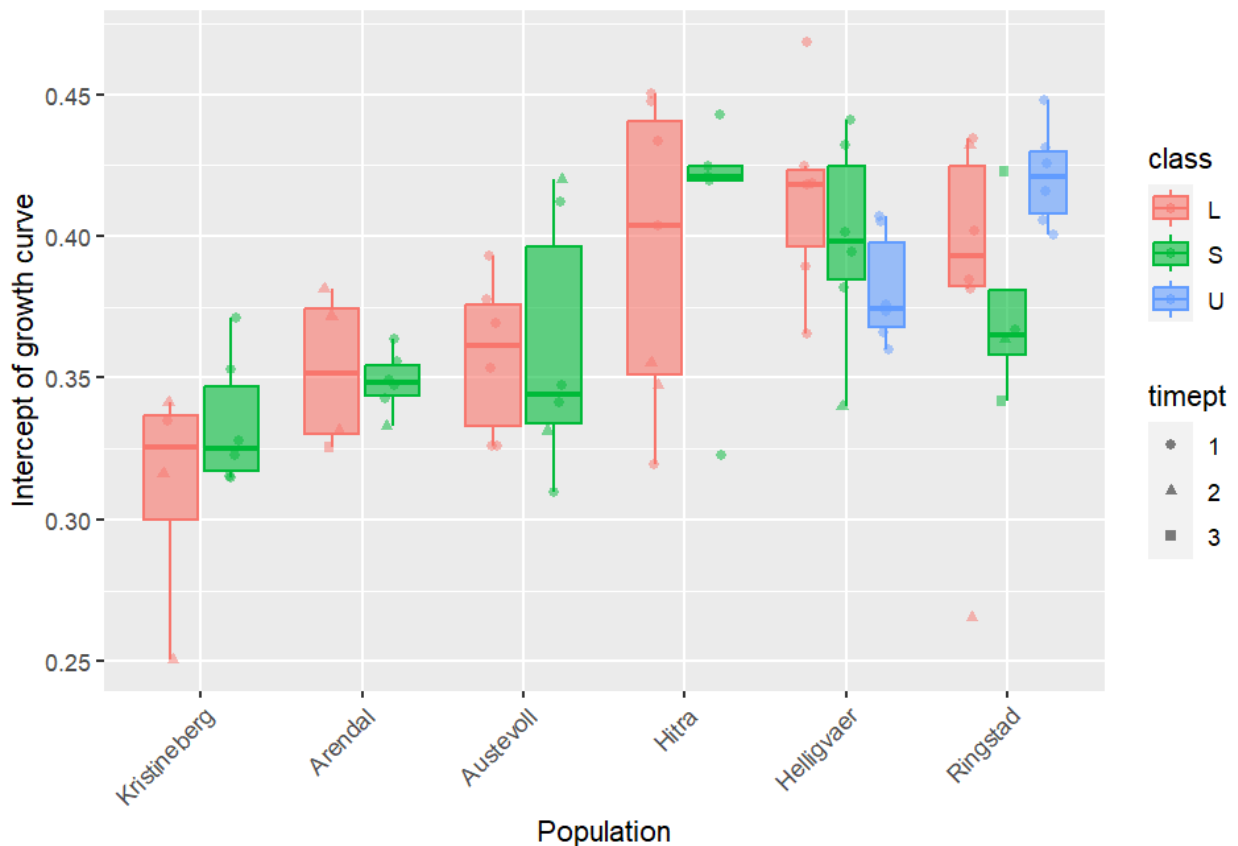
**Table 3:** Results from ANOVA test for difference in number of sclerites among all classes in Helligvær and Ringstad.

	<b>Sum sq</b>	<b>Df</b>	<b>F-value</b>	<b>p-value</b>
Class	2723,72	2	126,89	<b>&lt;0,001</b>
Residuals	311,2	29		

**Table 4:** P-value from post hoc pairwise comparisons in number of sclerites between all classes in Hellingvær and Ringstad. The classes are put into different groups depending on which class they are significantly similar to.

	<b>Group</b>	<b>Large</b>	<b>Small</b>
Large	B		
Small	A	<b>&lt;0,001</b>	
Unsexed	A	<b>&lt;0,001</b>	0,06713

### 3.4 Initial growth



**Figure 8: Initial growth for fishes from different populations and size classes.** Each color represents a size class: L=Large, S=Small, U=Unsexed. Each dot represents one individual fish where tree scales were sampled. The different figures on the dot represent the different timepoints when the fish were sampled. Circle= timepoint 1, Triangle= timepoint 2, Square= timepoint 3.

I wanted to see if there were any differences between the initial growth for fish from different populations and size classes, which is estimated by the distance between the first three sclerites after the focus. To do this, I used the intercept of the regression line calculated on the initial segment of growth trajectories (Figure 6), which represents the distance covered by the three first sclerites after the focus. Figure 8 shows a general pattern of increasing initial growth with latitude, and no clear differences between size classes. This is expected since initial growth should mostly be determined by environmental conditions, which all fish from the same population are expected to experience in the same way regardless of their size when sampled. Indeed, the statistical analyses confirm that there were significant differences between populations that support the increase of intercept with latitude (Table 5). A post hoc test allowed to attribute the different populations to groups that are summarized in table 6, and generally support a latitudinal increase. Size class did not significantly affect initial growth (table 5) and there was also not a significant effect of the interaction of location and class on initial growth.

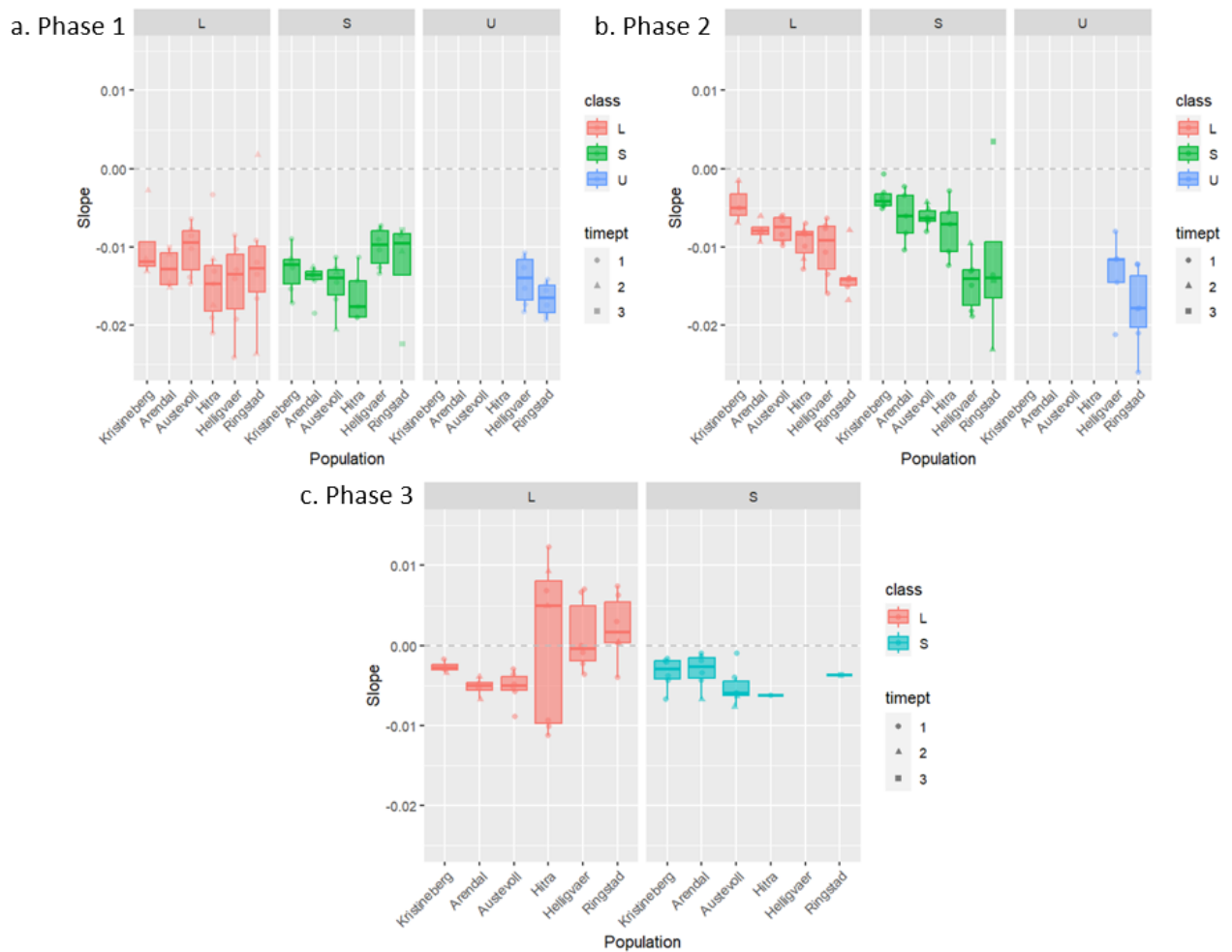
**Table 5:** Results from ANOVA test for difference in intercept of growth trajectory among populations and class.

	<b>Sum sq</b>	<b>Df</b>	<b>F-value</b>	<b>p-value</b>
Population	0,052040	5	6,8966	<b>&lt;0,001</b>
Class	0,000026	1	0,0172	0,896
Residuals	0,089041	59		

**Table 6:** P-value results from post hoc test to find which populations differed significantly from each other by the intercept of the growth trajectory. Population have the same group letter if they do not differ significantly from each other.

	<b>Group</b>	<b>Kristineberg</b>	<b>Arendal</b>	<b>Austevoll</b>	<b>Hitra</b>	<b>Helligvær</b>
Kristineberg	A					
Arendal	AB	0,68831				
Austevoll	AB	0,32273	0,99458			
Hitra	BC	<b>&lt;0,001</b>	0,05198	0,13318		
Helligvær	C	<b>&lt;0,001</b>	<b>0,01594</b>	<b>0,04534</b>	0,99747	
Ringstad	BC	<b>0,02984</b>	0,54287	0,81471	0,84979	0,60252

### 3.5 Growth



**Figure 9: Slopes of the growth trajectory of fish of the different size classes and populations.** a. The first 12 sclerites (phase 1). b. Sclerite 12-21 (phase 2). c. Sclerite 21-30 (phase 3). Each panel within a, b, and c represents a size class: L=Large, S=Small, U=Unsexed. Unsexed individuals have short growth trajectories so they are absent from phase 3. Each dot represents one individual fish where tree scales were sampled and averaged. The different shapes of the dot represent the different timepoints when the fish were sampled. Circle= time point 1, Triangle= time point 2, Square= time point 3.

I used the slope of the growth trajectories to see if there was any difference in the growth patterns for fish from different populations and size classes. I divided the growth trajectories/curves into 3 different phases based on sclerite numbers. Phase 1 was sclerite 1-12, phase 2 was sclerite 12-21, and phase 3 was sclerite 21-30 (Figure 5). The slope represents the changing growth speed over time, whereas a more negative slope means that growth speed decreases faster. The unsexed individuals were not included in the statistical model for the number of sclerites, since they only existed in two populations. We did however make a separate model to the subset of the data with only the two northern populations, where the tree classes were present (Large, Small, Unsexed), to see if there were any significant differences between classes in these populations, but no differences were found (Appendix B).

In Figure 8a we see that all slopes are negative which means that all individuals had a decrease in growth over time in phase 1. We conclude that the highest growth speed is in the

earliest stage. There were no significant differences between either class or location on the slope of the growth trajectory in this phase, which means the decrease of growth over time was the same for all populations and classes (Appendix B).

Phase 2 (Figure 9b) showed a strong latitudinal effect that was opposite to the results from the initial growth (Figure 7). Initial growth was higher in the North but the growth profile plunges down earlier, with a steeper negative slope, meaning that growth starts higher but decreases faster (see also Figure 6). The statistical analysis shows only the effect of population was significant, while classes did not differ in slope of growth trajectory (Table 7). It could be expected that classes did not differ in early slope because fish from within the same populations should experience the same growing conditions early in life. In particular, a post hoc test indicated that the slope of growth trajectory for Kristineberg, Arendal, and Austevoll was significantly different to Helligvær and Ringstad (Table 8). This means that the initial growth was higher in the North, but growth reduced faster in phase 2 for Northern populations. This may reflect environmental conditions, in particular that the fish in the northern populations are born closer to the end of the growing season.

**Table 7:** Results from ANOVA test for difference in slope of growth trajectory among populations and class for phase 2.

	Sum sq	Df	F-value	p-value
Population	0,00059707	5	8,2410	<0,001
Class	0,00000267	1	0,1846	0,6691
Residuals	0,00082594	57		

**Table 8:** P-value results from post hoc test to find which populations that were significantly different from each other by the slope of growth trajectory in phase 2.

	Group	Kristineberg	Arendal	Austevoll	Hitra	Helligvær
Kristineberg	B					
Arendal	B	0,60427				
Austevoll	B	0,51388	1,00000			
Hitra	AB	0,08160	0,89408	0,87457		
Helligvær	A	<0,001	0,02006	0,01062	0,17172	
Ringstad	A	<0,001	0,01339	0,00697	0,11291	0,99961

Phase 3 (Figure 9c) showed some Large class individuals in the 3 most northern populations having a positive slope, meaning that these fish increase their growth in this phase, which is not seen in any other parts of the growth profiles in this data (visible in Figure 6). There was no significant effect of either class or location on the slope in phase 3 (Appendix B), however, these distinct growth profiles are clearly visible in Figure 6, in the 3 northern populations, where some individuals show a clear restart of growth. This strongly suggests that two year old individuals exist in these populations, as a restart of growth would only be possible in spring or summer.

## 4 Discussion

By comparing populations at different latitudes, we aimed to determine if there was a latitudinal effect on two-spotted gobies life history strategies. The different climates across these latitudes can have a big impact on the fish, and by studying the variation between the different populations we hoped to gain valuable insights into how changing climates might affect them.

The results for total number of sclerites showed difference between the three most southern populations and the 3 most northern populations. The Southern populations had more sclerites for both the Large and the Small sized fish. This stood in contrast to my initial belief that bigger fish always meant more sclerites. The reason for fish in the North having fewer sclerites than fish from the south could have something to do with the longer winter in the North. Some of the scales had areas where the sclerites were very close to each other. These are called spring annulus or winter bands in other studies (Fukuwaka, 1998, Gilbert, 1913) and are a sign that the fish has lived through a winter (Appendix A). Many investigators have suggested that the annulus was formed during the winter because of less growth, but in 1990 Suzuki and Kaeriyama observed that Sockeye salmon (*Oncorhynchus nerka*) completely stopped their scale growth when the fish stopped their growth during starvation and winter. The annulus was thereby developed during a period of slow growth such as late fall and early spring (Fukuwaka, 1998). This understanding of annuluses is more consistent with my findings, since it would explain the smaller number of sclerites in Northern populations, even for very large fish. If sclerites were deposited continuously, even during periods without growth, no latitudinal effect on the number of sclerites would be found. Instead, long winters in the north give the fish from the northern populations a shorter time window for growth and they therefore do not have as much time to form sclerites as the fish from the southern populations. This can make the use of sclerite for ageing complicated or impossible when comparing populations from different locations but provides valuable information on the environmental conditions they experience. A fish with a certain amount of sclerites from Kristineberg is probably not the same age as a fish with the same amount of sclerites from Ringstad, since the fish in Kristineberg will have more time to create new sclerites than the fish from Ringstad. In conclusion, sclerite numbers may not represent calendar age, but instead represent the duration that the fish has spent growing.

There was also a significant difference between the Large and Small class fish in number of sclerite. This was especially clear in the Northern populations of Helligvær and Ringstad where the Large fish had almost double the amount of sclerites than the Small fish. This could indicate that the Large fish are indeed a year older than the Small fish in these populations, since it does not seem likely for them create the double amount of sclerite in the same climate within one year. Some studies have concluded that there is no correlation between the number of sclerites and age (Fouda, 1979), but others say that since the sclerite deposits regularly over time, it can be a good way of determining age (Das, 1994). In 1975, Hesthagen reported that relatively more Common gobies (*Pomatoschistus microps*) in the Oslo fjord reached an age of 2 years, than in southern populations (Hesthagen, 1975). It has also been suggested that two spotted gobies that did not reach maturity in the first year,



would do so the following season (Johnsen, 1945). Our results support this observation and the hypothesis of there being 2 year olds in the Northern populations. The absence of a bimodal distribution in the south means that if 2 years old exist in the south, they are rare. Although there is no significant interaction between class and populations, our figure shows that the difference in number of sclerites is not as big in the South, so it is not clear that these Large individuals are 2 years old. The reason for Large class individuals in the South having more sclerites than Small class individuals, could therefore be caused by the Large individuals being born earlier in the same season than the Small individuals. For example, a Large fish could be born in May while a Small fish was born in July and the difference in sclerite number would be explained by 2 months of additional growth.

The intercept of the linear fit to the growth curve in Phase 1 estimated the initial growth of the fish from the different populations and classes. Here we saw again that the 3 southern populations and the three northern populations formed two distinct groups. The border between the groups was not as clear as for sclerite numbers since Arendal and Austevoll were also not different from Ringstad. The intercept was higher for the Northern populations, which means that the fish in the North grew faster in the early stages than the fish in the South. One explanation for this could be counter gradient adaptations. This means that the fish in the North could have adapted by for example utilizing the resources differently in colder conditions and thereby allowing them to grow more, or having developed specific traits that enhance growth in colder conditions (Conover and Present, 1990). As mentioned in the introduction, there is usually a strong correlation in fish between the size of the juveniles and their survival during winter. Consequently, fish from the northern population that must face a longer winter period would benefit from being larger, but why don't fish from the south do the same thing? It could be because of the cost of fast growth. When utilizing their resources for growth it means that they must invest less in things like their immune system or swimming capacity, and this cost might not be worth paying for fish in the South. For example, in the Atlantic silverside (*M. mendina*) demonstrated a trade-off between growth and swimming performance, with fast-growing fish having inferior swimming capabilities than slow-growing fish (Conover and Present, 1990). In general, fast growth may enhance fitness through the benefits of attaining a large body size quicker, but when fast growth means that they have to trade on other important properties such as immune systems or swimming capacity it may not be worth the cost for some populations. A weaker immune system can lead to the fish being more prone to viruses or getting sick from parasites, which are more likely to occur in denser southern populations, and a lower swimming capacity can make the fish lower their escape success from predators (Korfel et al., 2015, Arnott et al., 2006).

The fact that we see a trend in both the number of sclerites and the initial growth where the 3 southern populations are significantly different from the 3 northern populations could indicate that the fish are affected by the different climates at the different latitudes. Since our results separates our populations into two groups with the 3 southern populations (Kristineberg, Arendal, and Austevoll) and 3 northern populations (Hitra, Helligvær, and Ringstad) it could mean that the climate for these populations is similar to each other, and that the fish within these groups has therefore adapted in the same way.

The slope of growth trajectory in Phase 2 was steeper for the 2 most northern populations than for the 3 southern populations. The slope shows the change of growth over time, so the steeper slope in the populations in Helligvær and Ringstad have a faster change in growth in a negative direction than the southern populations in this phase. A steeper negative slope could mean that the fish were born closer to the end of the growing season, since the growth rate naturally slows down as winter is approaching, resulting in the formation of the annulus (Appendix A). This is consistent with the fact that the breeding season starts later and ends earlier at higher latitudes, thereby allowing offspring in the north less time for growth before winter. As mentioned in the result section, this latitudinal effect is opposite to the results from the initial growth, where the growth is higher in the north. In summary, Northern fish start with faster growth, which could be a sign of counter gradient adaptation, but their growth speed declines faster.

In phase 3 we observed no significant effect of population or class. However, it is visible in Figure 3 that the Large individuals in the Northern populations have a positive slope in that phase, meaning that they increase their growth. The increase in growth is also visible on the scale, where you can see that the intersclerite distance gets bigger after the annulus. The scales that show the increase in growth also have two spring annuluses (Appendix A). The fish in the southern populations have a negative slope. The reason for there not being a significant effect of populations in this phase could be due to the low number of individuals in this phase. The increase in growth in the northern populations is also very clear from the growth profiles in Figure 6, with the exception of Hitra, where some of the Large individuals show the increase in growth indicative of a second year, while others do not. This could be because Hitra is in the middle of our latitudinal study and that the climate allows for both life history strategies to coexist. This result supports the hypothesis of there being 2 years old fish in the North, however it remains unknown whether these individuals reproduce two consecutive years, or simply postpone their reproduction to the second year. The restart in growth suggests that they don't reproduce in the first year but put all their energy into growth. Fish that spawn use a lot of energy for this, and therefore would have a low growth rate (Miller, 1975). This is also the only instance in the data where the slopes of growth trajectories become positive.

Scale analyses are a common way to look at age determination, growth estimation, and spawning in salmonids (Fukuwaka, 1998, White and Medcof, 1968). While the scales of two-spotted gobies contain less information compared to those of salmonids, due to their shorter live and only living in salt water, their scales still offer a lot of intriguing data. Scale analysis of two-spotted gobies can be used for age determination with the spring annuluses, or when comparing the number of sclerites within a location. However, great care should be taken when comparing individuals from different locations. The distance between the sclerites can be used to understand the growth pattern of the fish. This was also done by Miller in 1975 for the Common goby (*Pomatoschistus microps*) and the scales from this study appear very similar to the scales of the two-spotted goby, but in addition to having spring annuluses, these scales also had summer annuluses that showed a decrease in growth rate during the spawning season. This is not something that we recorded for the two-spotted goby in this project.

In addition to scale analysis, researchers also use otoliths to determine the age of salmonids and other gobies such as the Rock goby (*Gobius paganellus*) and the Giant goby (*Gobius cobitiis*) (Fukuwaka, 1998, Miller, 1961, Gibson, 1970). During the start phases of this project, we tested this with the two-spotted goby, but their otoliths turned out to be unsuitable for age determination because they did not show any clear annual rings. This was the same conclusion that Collins came to in 1981 (Collins, 1981).

The sum of our results, with the difference for initial growth and number of sclerites between the Southern and Northern populations, coupled with the observed growth patterns, indicate a noticeable latitudinal effect on the two-spotted goby. The higher initial growth observed in the northern populations, despite the less favorable growing conditions, implies an adaptive response aimed at enhancing their chances of winter survival. Determining whether this adaptation stems from local selection pressures or phenotypic plasticity, wherein traits that are heritable play a role, would need a garden experiment to see if the fish are behaving according to the environment they are put in or not. Notably, other species have been enhanced growth rates at higher latitudes, such as juvenile turbot (*Scophthalmus maximus*), which have demonstrated counter gradient adaptation (Salvanes et al., 2003).

From the scale analysis performed during this project, we have learned that two-spotted gobies in the north have a shorter growing season than individuals from the south, yet they are able to become bigger. This is done by higher initial growth and by a biannual cycle instead of an annual cycle, which allows them to increase their growth in their first year. A higher growth rate in starting phases has also been demonstrated for Atlantic silversides, juvenile Turbot, and anadromous Arctic charr (*Salvelinus alpinus*) from northern populations (Salvanes et al., 2003, Conover and Present, 1990, Chavarie et al., 2010), but a change from annual to a 2 year life cycle has not been very well documented in other species. A study on the copepod *Eucalanus bungii* suggested that a multiple-year cycle could occur, ranging from less than a year to 3 years, based on environmental variations (Miller et al., 1984, Tsuda et al., 2004). An increased longevity for Sandgobies (*Pomatoschistus minutus*) with increasing latitude has also been noted, where some of them reached the age of 2 years (Hesthagen, 1975). The lack of documented cases of species changing their life history strategy from an annual cycle to a 2 year cycle indicates that this is either a very rare occurrence or that not many studies has looked at it.

This study uncovered significant differences in growth patterns and sclerite formation between Northern and Southern populations of two-spotted gobies. This indicates a latitudinal effect, where the northern populations have adapted to the colder climate. The difference in the number of sclerites between Large and Small individuals and the positive slope in phase 3 in the north, along with the size distribution, are also strong indicators of being 2 years old in the northern population.

One of the things that would be interesting to have a second look at, is the breeding strategies of the two years old. Do they breed only in the second season, or are they also able to breed in their first season? Our result suggests that most of them only breed in their second season, but from the growth trajectory it could look like some of them breed in to season, but at this stage this is only speculation. It would also be interesting to learn more about the unsexed

individuals, and if these do become two years old. Given that they do not show any secondary sexual characters, it is likely that they are postponing reproduction to the following year. However it is not clear whether all 2 year old fish have been following that strategy in the previous year. Common garden experiments could also confirm how fish are different in growth, by for example putting fish from southern populations in tanks simulating the northern climate or the other way.

## Conclusion

This study conducted on two-spotted goby across different latitudes revealed significant insights into the species life history strategies and their adaptation to varying climates. The examination of populations at different latitudes demonstrated clear differences in sclerite formation and growth patterns, indicating a latitudinal effect on these fish.

The results suggest that the different seasonality at higher latitudes influence the growth and development of two-spotted gobies. Fish from Northern populations exhibited fewer sclerites and higher initial growth compared to those from Southern populations, indicating adaptations to colder conditions. The positive slope in the growth trajectory for Northern populations in Phase 3 further supports the hypothesis of 2 years old fish, allowing for increased growth in the first year. These findings challenge conventional understanding of the species life cycle, suggesting a transition from the mainly annual cycle to a mainly biannual one in the north as a response to environmental pressures.

Further investigation into the breeding strategies of two-year-old individuals is warranted. Understanding whether they only breed in their second year or are capable of breeding in their first year would shed light on their reproductive dynamics. Additionally, exploring the fate of Unsexed individuals and their potential to become two years old could provide valuable insights into the factors influencing the different life history strategies within populations. Furthermore, conducting experiments to simulate northern climates on fish from Southern populations, and vice versa, could lead to more knowledge about the different life history strategies.

To conclude, this study highlights the importance of understanding fish adaptation to changing climates, with this great demonstration of adaptation. The findings contribute to our understanding of how environmental variations influence fish populations and emphasize the need for further research on the life history strategies at varying climates.

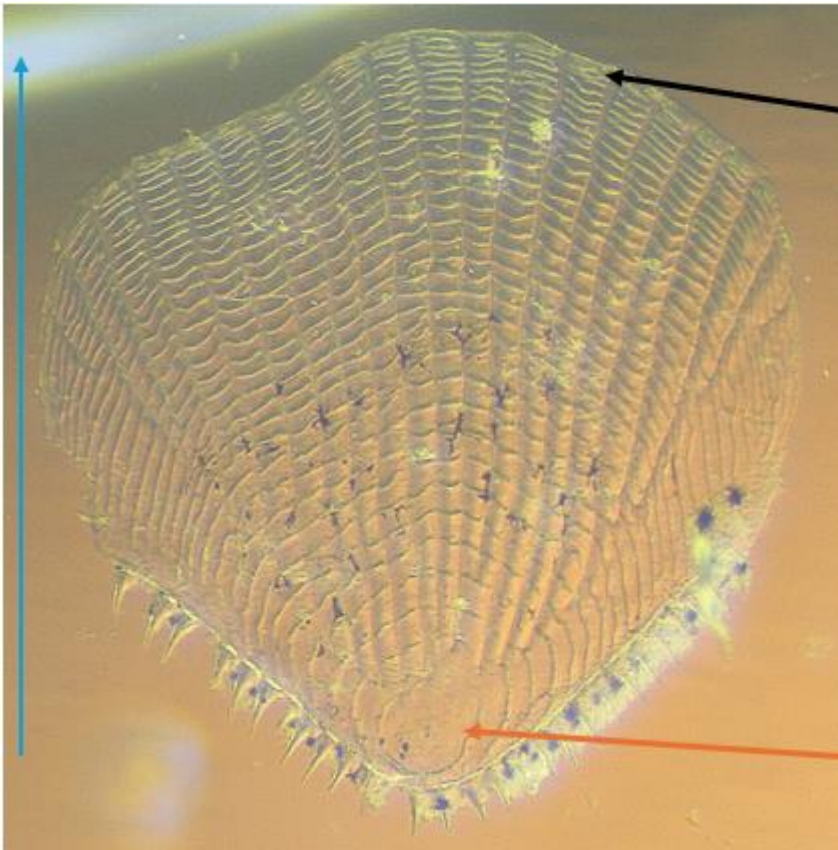
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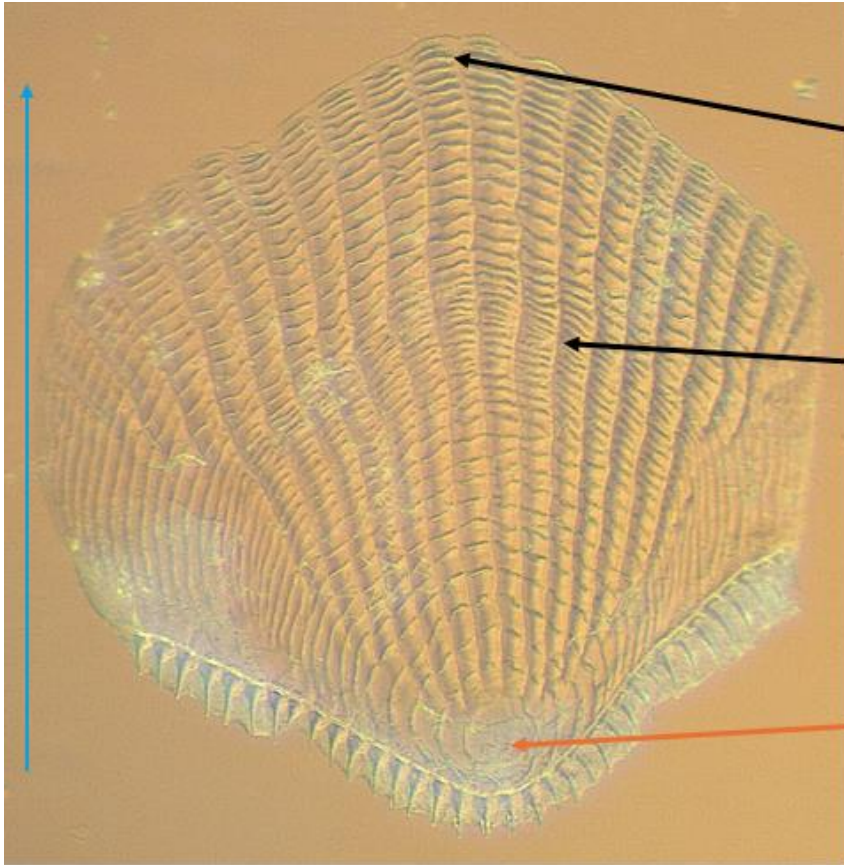
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## Appendix A: Scales

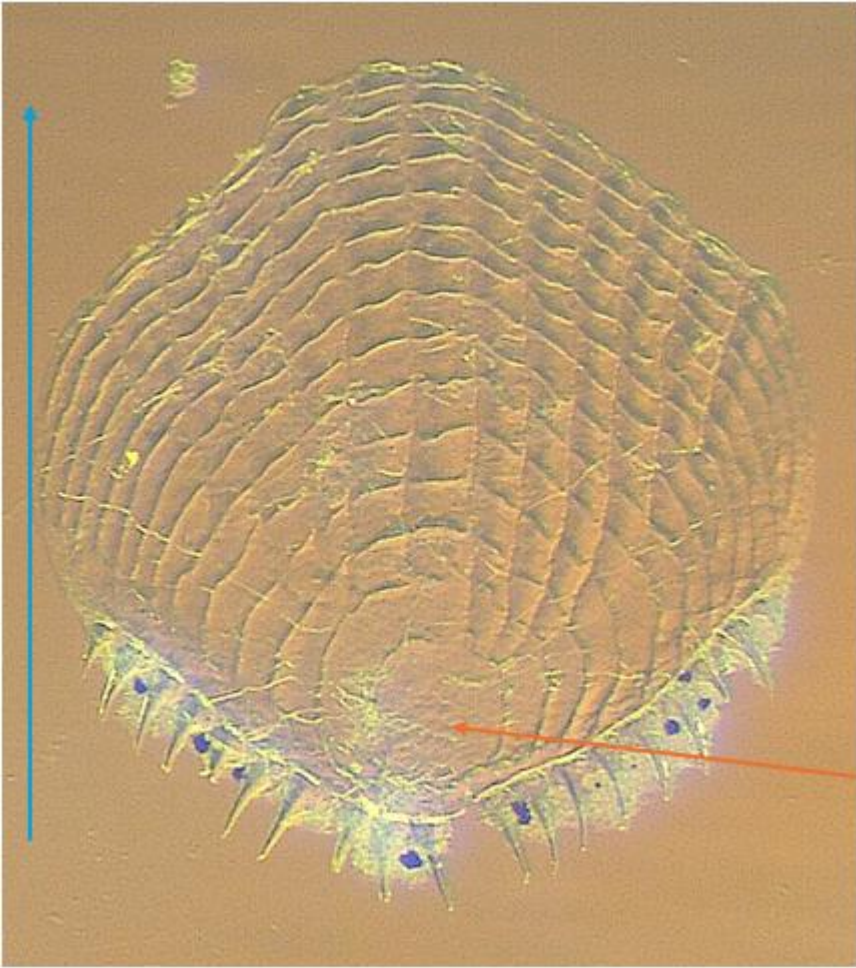


**Figure 4: Scale with one annulus.** This figure shows a scale with only one annulus, which means that it belongs to a 1 year old fish. The black arrow points to the area of the annulus, which is where the sclerites are very close to each other. The red arrow points to the focus, which is where the first sclerites are made. The blue arrow points in the direction sclerites grow, showing that the sclerites closest to the focus are the oldest, while the ones closest to the end are the newest.





**Figure 2: Scale with two annuluses.** This figure shows a scale with two annuluses, which means that it belongs to a fish that is likely to be 2 years old. The black arrows point to the area of the annuluses, which is where the sclerites are very close to each other. The red arrow points to the focus, which is where the first sclerites are made. The blue arrow points in the direction sclerites grow, showing that the sclerites closest to the focus are the oldest, while the ones closest to the end are the newest.



**Figure 3: Scale from an unsexed fish.** This figure shows a scale from an unsexed fish. It is smaller and has fewer sclerites than scales from Large or Small fish. The red arrow points to the focus, which is where the first sclerites are made. The blue arrow points in the direction sclerites grow, showing that the sclerites closest to the focus are the oldest, while the ones closest to the end are the newest.

## Appendix B: ANOVA table for slopes with no significant differences

*Table 1: Results from ANOVA test for difference in slope among populations and class for phase 1.*

	<b>Sum sq</b>	<b>Df</b>	<b>F-value</b>	<b>p-value</b>
Population	0,00009869	5	0,7710	0,5745
Class	0,00001674	1	0,7196	0,3997
Residuals	0,00137265	59		

*Table 2: Results from ANOVA test for difference in slope among all classes in Helligvær and Ringstad for phase 1.*

	<b>Sum sq</b>	<b>Df</b>	<b>F-value</b>	<b>p-value</b>
Class	0,00010974	2	2,0635	0,1441
Residuals	0,00082432	31		

*Table 3: Results from ANOVA test for difference in slope among all classes in Helligvær and Ringstad for phase 2.*

	<b>Sum sq</b>	<b>Df</b>	<b>F-value</b>	<b>p-value</b>
Class	0,00008683	2	1,4492	0,2507
Residuals	0,00089881	30		

*Table 4: Results from ANOVA test for difference in slope among populations and class for phase 3.*

	<b>Sum sq</b>	<b>Df</b>	<b>F-value</b>	<b>p-value</b>
Population	0,00023584	5	2,1978	0,07067
Class	0,00000587	1	0,2734	0,60360
Residuals	0,00098722	46		

*Table 5: Results from ANOVA test for difference in slope among all classes in Helligvær and Ringstad for phase 3.*

	<b>Sum sq</b>	<b>Df</b>	<b>F-value</b>	<b>p-value</b>
Class	2,6595e-05	1	1,4829	0,2488
Residuals	1,9728e-04	11		

