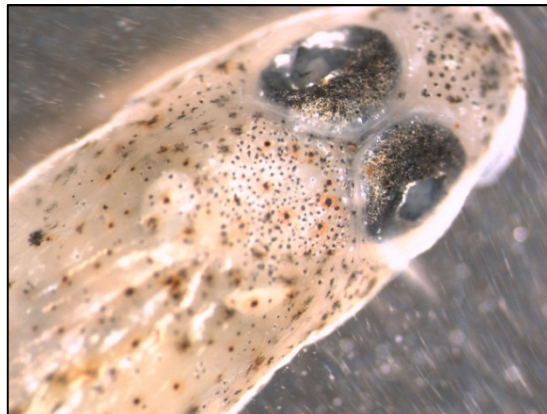# Species separation and population structure of three Gobiidae (*Pomatoschistus microps*, *Pomatoschistus minutus* and *Pomatoschistus pictus*) using otolith shape and fish condition

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ABSTRACT

Population structure and species separation in three species of the genus *Pomatoschistus* (*P. microps*, *P. minutus* and *P. pictus*) was studied using otolith shape and morphology and fish condition. Using a 1 m-wide beam trawl ca 2000 gobies were collected in several locations across the European Atlantic coast.

Pomatoschistus minutus showed a much higher condition factor than the remaining 2 species. In the intraspecific analysis *P. microps*' population from Skibotn/Sørbotn showed the highest K_{rel}, followed by the populations from Minho while in the case of *P. minutus* the population from Texel showed by far the highest Kr_{el}. In both species the animals collected during the summer season had the highest K_{rel}, with the winter season scoring the lowest.

In the morphological analysis the three species showed the same tendency in all the comparisons: the bigger the total length, the bigger the area, Feret's diameter and otolith weight. Also, the bigger the area, the bigger the perimeter. Contrarily, when the animal was bigger the otolith was less circular and there was a higher range in the otolith's circularity. In general *P. pictus*' and *P. microps*' otoliths have approximately proportional relationships between the variables while *P. minutus*' otoliths show a considerable difference: proportionally *P. minutus*' otoliths not only tend to be heavier in larger specimens but also larger and less circular than otoliths from the common and the painted goby. The sand goby also shows higher values of Feret's diameter. At all sizes when the painted goby has proportionally smaller and more circular otoliths than the common goby. In the intraspecific comparisons the different populations of both *P. microps* and *P. minutus* showed the same relationships between the variables as described for the species.

When using all the variables combined it was not possible to clearly separate the species based on otolith shape and morphology, although the degree of separation was higher when all the populations were included. In the intraspecific analysis *P. microps*' populations from Minho and Skibotn/Sørbotn could be separated. The remaining population, as well as all the populations from P. minutus, showed a high degree of overlapping.

Keywords: *Pomatoschistus*, otoliths, intra- and interspecific variation, fish condition

I.   INTRODUCTION

The family Gobiidae is one of the largest families of fish spread throughout the tropical and temperate seas (Nelson 1994). They are not only abundant in numbers but also very diverse in the type of habitats they live in: pelagic or demersal habitats, sandy, rocky and/or muddy bottom. They can be both amphidromous or complete their life cycle entirely in freshwater, brackish or marine environments (Hoese 1984, Miller 1986, Nelson 1994, Froese & Pauly 2000, Huyse et al. 2004, Guelinckx 2008, Larmuseau et al. 2009b). Among the several genus of Gobiidae occurring in European coasts, estuaries and lagoons is the genus *Pomatoschistus*. *Pomatoschistus microps* (Krøyer, 1838), also know as the common goby, and *Pomatoschistus minutus* (Pallas, 1770), known as the sand goby are two conspicuous species with recognized importance along the European Atlantic coast and inner seas, estuaries and lagoons. Both the sand and the common goby are abundant throughout their distributional range representing an important component of those ecosystems (Miller 1963, Webb 1980, Arruda et al. 1993, Bouchereau & Guelorget 1997, Dolbeth et al. 2007) and playing an essential role as intermediate predators in the estuarine food-webs (Reise 1977, Doornbos 1984, Moreira et al. 1992, Leitão et al. 2006). Several authors have demonstrated that *P. microps* and *P. minutus* serve as prey to a few different species, some of them with commercial value, like the cod *Gadus morhua*, the twaite shad *Alosa fallax* or the European eel *Anguilla anguilla*, but also the brown shrimp *Crangon crangon*, the tub gurnard *Trigla lucerna* and some birds like *Egretta garzetta* and *Calidris alpina*, just to name a few (Magnhagen 1990, Lindström & Ranta 1992, del Norte-Campos & Temming 1994, Salgado et al. 2004). With a lifespan of 1-2 years (varying with latitude) and attaining sexual maturity in less than one year (Bouchereau & Guelorget 1997) both the sand and the common goby are excellent models for the study of reproductive outcome (e.g. J.-L. Bouchereau et al. 1989; Kvarnemo & Forsgren 2000), egg development (e.g. Fonds & Van Buurt 1974), adaptation to different conditions (e.g. Dolbeth et al. 2007; Pampoulie et al. 2000), behavior (e.g. Kvarnemo et al. 1998; Lissåker et al. 2003) and growth (e.g. Fouda & Miller 1981; Arellano 1995) among others (Stefanni et al. 2003, Berrebi et al. 2006, Larmuseau et al. 2007, Larmuseau et al. 2008, Larmuseau et al. 2009a). Being extremely similar in their morphology and life cycle these two species also show some differences. It is important to analyze how are the similarities and differences between them contributing for the current patterns in population structure and species differentiation.

Up to this moment there are a few unanswered questions regarding these two species. It is known that individuals of *P. microps* and *P. minutus* tend to occupy different areas in the estuaries reducing the potential for competition among them (Evans & Tallmark 1985, Pampoulie et al. 2001, Leitão et al. 2006). What remains unclear is to what extent the distribution along the estuarine gradient and the different adaptations in the local life cycles are due to the interaction with other species, if they are shaped by local environmental requirements or both (Thorman 1982, Thorman & Wiederholm 1983, Wiederholm 1987, Lindström & Ranta 1992, Leitão et al. 2006).What also remains uncertain is if there are driving forces shaping the migratory paterns within estuaries and between estuaries and coastal areas other than temperature and reproduction. Several hypothesis are put forward: (1) competition for nest sites (Bouchereau et al. 1991, Nellbring 1993, Pampoulie et al. 1999), (2) existence of individual trade-offs between environmental cues and stage-dependent survival rates (Fonds & Van Buurt 1974, Bouchereau et al. 1991) or (3) different predation pressure and competition for food that turn this migration into a facultative process (Evans & Tallmark 1984, Magnhagen 1985, Lindström & Ranta 1992). It is known that temperature plays a key role triggering these migratory processes and so it is expected that they will be altered due to climate change (Guelinckx et al. 2008a, Larmuseau et al. 2009b). However there are still no studies confirming this hypothesis.Also lacking are common-garden experiments to study growth rates: it is uncertain if there is variation among populations, if there are differences between sexes (particularly during the breeding season) and how these are influenced by photoperiod. Ultimately there is still the need to evaluate if there is a "latitudinal compensation" (Levinton 1983) on different aspects such as food conversion, growth rates, length of the spawning season, reproductive outcome, among others.

So far only a few studies focused on the otoliths of these species (Arellano 1995, Geffen et al. 1998, Coelho 2005, Guelinckx 2008) but only one studied their shape (Assis 2000).

In order to investigate if *Pomatoschistus microps* and *Pomatoschistus minutus* could be distinguished using the shape of the otoliths and if it was possible to distinguish populations using the same method about 2000 fish were sampled for this study. These animals belonged to both species mentioned beforehand but also to the related species *Pomatoschistus pictus*.

1.1 SPECIES DESCRIPTION

Pomatoschistus microps (Figure 1) is an estuarine species particularly abundant in shallow waters across the European Atlantic coast.
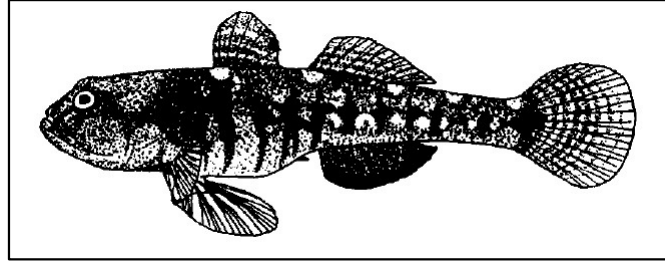
Figure 1: *Pomatoschistus microps* (Fonds & Veldhuis 1973)

It occurs in an extensive geographic area, from Norway, around the British and Irish coasts, to Morocco, including the Baltic Sea. It is also present in lagoons and estuaries of the western part of the Mediterranean Sea, as well as in the Canary Islands and Mauritania, within the latitude parallels of 20ºN and 69ºN (Mediterranean, temperate and cold climatic zones) (Miller 1986, Bouchereau & Guelorget 1997) (Figure 2).
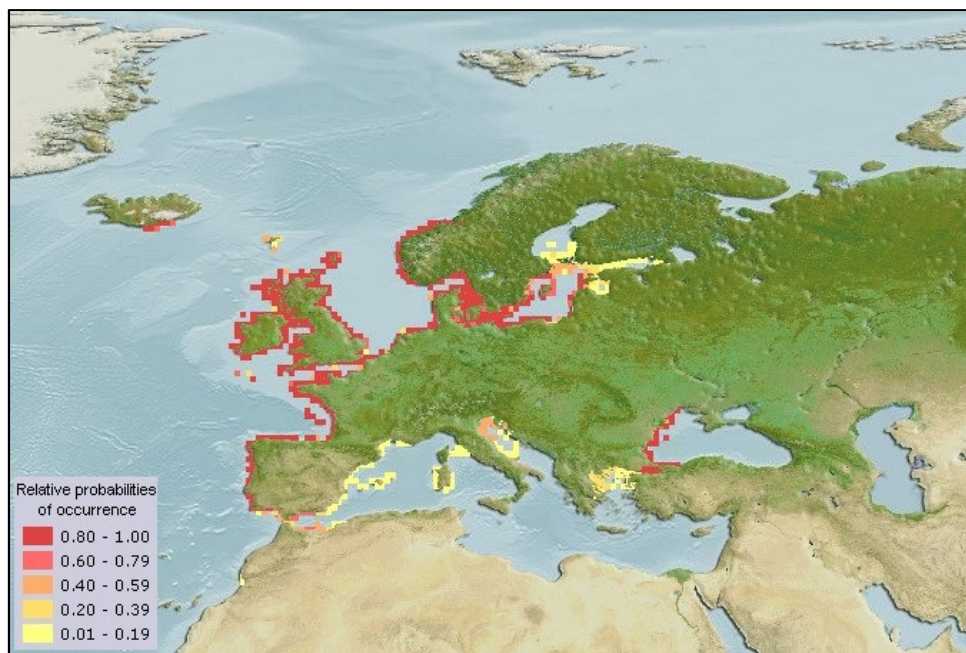
Figure 2: *Pomatoschistus microps*' distribution (Kaschner et al. 2010a)

Closely related to the common goby is *Pomatoschistus minutus* (Figure 3). It has a very similar distribution, reaching high densities throughout its distributional range: in the north-

eastern Atlantic from near Tromsø, Norway, ca 69ºN, and around the Faroe Islands, to the south of Spain, ca 35ºN;
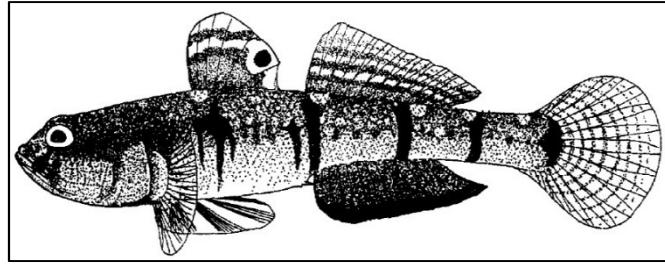
Figure 3: *Pomatoschistus minutus* (Fonds & Veldhuis 1973)

It is also present around the British Isles and in a more fragmented pattern in the Baltic Sea to the southern Gulf of Bothnia, the Gulf of Finland, the western Mediterranean (Gulfs of Lions and Genoa), in the Adriatic (Venice Gulf) and in the western Black Sea coast (Figure 4) (Webb 1980, Arellano 1995).
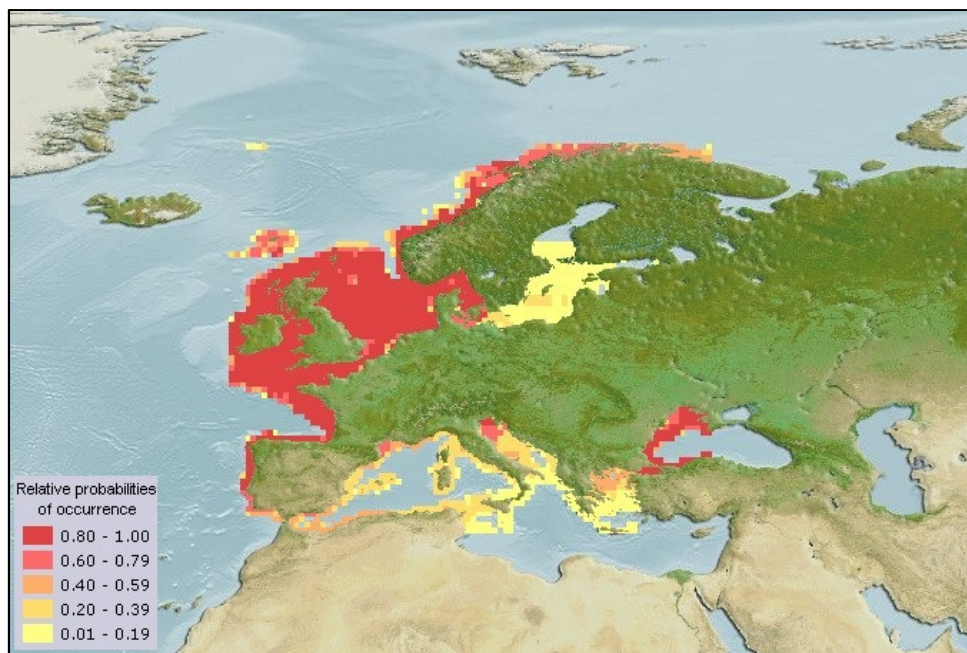
Figure 4: *Pomatoschistus minutus*' distribution (Kaschner et al. 2010b)

This widespread distribution in both species is mainly related with their tolerance to a wide range of temperature and salinity values (and more likely with the interaction of the two factors) (Fonds 1973, Fonds & Van Buurt 1974, Leitão et al. 2006) although other factors have to be considered such as food density (e.g. Dolbeth et al. 2007), presence of predators

(e.g. Lindström & Ranta 1992) or suitable breeding substrate (e.g. Nellbring 1993), just to name a few. Both species preferentially occupy shallow soft bottoms like bare mud or sandy areas but can also be found in the densest vegetated areas (Tallmark & Evans 1986).
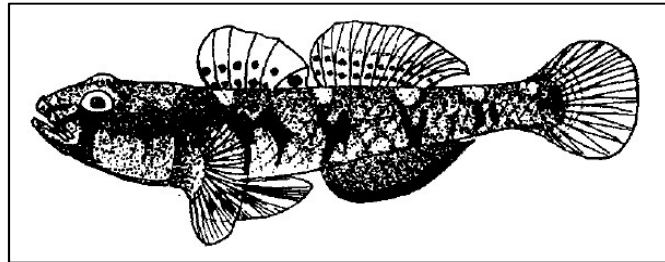
Figure 5: *Pomatoschistus pictus* (Fonds & Veldhuis 1973)

Pomatoschistus pictus (Malm, 1865) (Figure 5), commonly known as the painted goby, is a small fish up to 60 mm length.
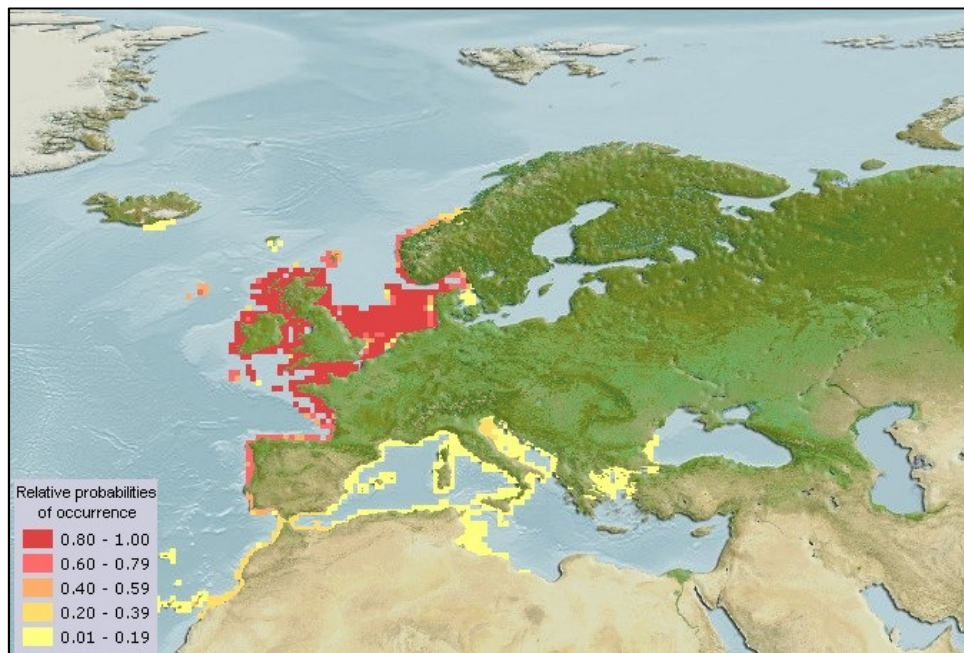
Figure 6: *Pomatoschistus pictus*' distribution (Kaschner et al. 2010c)

It is also present in the European Atlantic coast from Trondheim to Spain, Canary Islands and being sometimes reported to exist also in the Mediterranean and Black Seas (Miller 1986, Bouchereau 2003) (Figure 6). It lives on sandy or coarser bottoms from 1 to 55 meters depth. As the previous two species it feeds mainly on amphipods and copepods (Miller 1986). Its distinct characteristics are: dorsolateral eyes, two dorsal fins with rows of black

spots, four pale saddles on the back, four dark double spots on the sides, absent papillae between the eyes, rows of dark brown-black spots in the dorsal fin which are interspersed with bands of iridescent blue and pink (Froese & Pauly 2000, Bouchereau 2003).

P. minutus vs P. microps

These three *Pomatoschistus* species are extremely similar in their morphology and life cycle. *Pomatoschistus minutus* and *Pomatoschistus microps* are the two species that are the most similar. It is therefore important to clarify which are the similarities and differences between them in order to identify potential sources of (lack of) phenotypic plasticity. They have been originally separated based on their differing morphology by Boulexger (Boulexger 1911) (using scales, fin formulae, shape and colour) and Fage (Fage 1914) (using the sensory papillae), although posteriorly Petersen (Petersen 1916) has described both species together under the name *Gobius minutus* but still recognising the existence of two different forms with different number of vertebrae (33 and 31, most likely corresponding to *P. minutus* and *P. microps*, respectively). Since then there have been confusing identifications with these species being recurrently grouped and treated as *Pomatoscshitus* spp. (Nellbring 1986, Pasquaud et al. 2004, Ehrenberg et al. 2005). Generally the common and the sand goby are readily distinguished by the presence of a dark spot in the base of the pectoral fin on the former as well as by the different pigmentation on the base of the caudal fin (Webb 1980, Arellano 1995). The common goby has a T-shaped black spot while the sand goby has a triangular-shaped spot in the same place (personal observation). Nevertheless identifications based merely on colour/pigmentation should be handled with care. When in the lab the pattern of the sensory papillae is the most reliable characteristic to use for the proper identification of the specimens (Webb 1980).

Both species have an extremely similar life cycle, occurring simpatrically in shallow soft-bottoms. Differences in the life cycle of these two species are only recognizable during the reproductive season. Each individual might spawn only once or several times during the season either in spring, summer, autumn and/or winter depending on the location (and therefore the local temperature pattern) of the population (Bouchereau & Guelorget 1997). Their high plasticity to environmental conditions (Bouchereau 1997, Dolbeth et al. 2007) leads to differences in the life cycle throughout their distributional range: Mediterranean populations show a "contracted" life cycle with fast growth and rapid maturity, increased spawning and shorter lifespan (Bouchereau 1997) while northern Atlantic populations display

a "protracted" life cycle where lifespan is extended but the spawning season is shorter (Healey 1972, Fonds 1973). The Portuguese populations have shown intermediate characteristics between the Mediterranean and northern Atlantic populations (Arruda et al. 1993, Leitão et al. 2006). As eurytopic species *P. minutus* and *P. microps* can be found at salinities that vary between 5‰ and 35‰ and temperatures between 1-40ºC, although this tolerance might vary with the life stage and the geographic location of the stock (Fonds 1970, Fonds & Van Buurt 1974). When different values of salinity and temperature are combined their tolerance is also altered; some authors have suggested that this change in their survival is related not with temperature and salinity tolerance itself, but with differences in the availability of oxygen that they might lead to (Fonds 1973, Fonds & Van Buurt 1974, Leitão et al. 2006). Nevertheless, some differences are found in their tolerance: *Pomatoschistus microps* seems to be a euryvalent species capable of enduring a broader spectrum of salinity and temperature combinations, while *P. minutus* has a less wide range of salinity and temperatures that it can live on (Fonds & Veldhuis 1973, Fonds & Van Buurt 1974, Bouchereau 1997). These differences in temperature and salinity tolerance give rise to small differences in the life cycle and distribution within the estuaries (Fonds 1973, Fonds & Van Buurt 1974, Bouchereau et al. 1989, Bouchereau 1997, Leitão et al. 2006): in general *P. microps* is found throughout the estuaries although it reaches higher densities upstream while *P. minutus* has higher marine influence (Fonds & Van Buurt 1974, Dolbeth et al. 2007).

Some authors have also reported different sediment preferences in these 2 species. It is argued that these preferences vary with the density of the populations and are correlated with predator avoidance. When densities of both species are high spatial segregation between the sand and the common goby can be seen whereas when densities are lower there is an overlap in the type of substrate chosen (Edlund & Magnhagen 1981, Magnhagen & Wiederholm 1982). Also, when predation pressure is higher both species alter their behaviour and consequently the type of substrate they choose also changes, alternating between silty sandy areas (Malavasi et al. 2005) or between bare and vegetated areas (Wiederholm 1987). The sand goby is usually found at depths up to 20 m but can also occur up to 60-70 m depth, while the common goby is found in more shallow areas from 1 m to about 12 m depth (Doornbos & Twisk 1987).

1.2 BIOGEOGRAPHY AND POPULATION STRUCTURE

The current patterns in species distribution can be explained by speciation and extinction events, glaciation periods and the consequent variations in sea level but also by the species' tolerance to a number of factors such as temperature, salinity and dissolved oxygen (Gysels et al. 2004a, Gysels et al. 2004b, Soberon & Peterson 2005, Larmuseau et al. 2009b). Gobiidae includes small vertebrates that have as a characteristic a fused pelvic fin shaped as an adhesive disc which as a consequence turns gobies into poor swimmers. Nevertheless they are capable of withstanding moderately strong currents (Miller 1986). Despite the unequivocal allocation of these species as Gobiidae their systematic relationships with other gobioids based on morphology remains unclear, with a sister group apparently from the Indo-Pacific region (McKay & Miller 1997, Huyse et al. 2004). Although the taxonomy of species belonging to the genus *Pomatoschistus* is well resolved the high level of morphological and ecological similarity results in recurrent misidentifications. At present, identification keys use morphological characters that are applicable in the identification of adults and late juveniles. Larvae, post-larvae and damaged individuals are not possible to identify using only identification keys thus *P. minutus*, *P. microps*, *P. norvegicus* (Collett 1903), *P. lozanoi* (de Buen 1923) and *P. pictus* (Malm 1965) are repeatedly put together and treated as *Pomatoschistus* spp. (see for example Bardin & Pont 2002; Nellbring 1986).

The population structure of highly vagile marine animals is dependent on several factors. In the specific case of these gobies the factors influencing the most the maintenance (or lack) of gene flow between the populations are: (1) the existence of a relatively short phase where eggs and larvae are planktonic (Fonds & Van Buurt 1974, Bilton et al. 2002), (2) the reproductive behavior (with or without migrations) (Bouchereau & Guelorget 1997), (3) past climatological and vicariance events (Gysels et al. 2004b, Larmuseau et al. 2009b) and (4) oceanic and coastal currents (mainly North-Atlantic Current (NAC), Shelf Edge Current (SEC), Fair Isle Current (FIC), East Shetland Atlantic Inflow (ESAI), and tidal currents which are of particular strength in the North Sea and the English Channel) (Turrell 1992, Gysels et al. 2004b). Particularly important is the Almería-Oran Front which is said to form a barrier to several marine species and therefore reducing the gene flow between Mediterranean and Atlantic populations, and the southern Adriatic gyre which may have strong influence in the isolation of the Adriatic populations (Figure 7) (Tintore et al. 1988, Gysels et al. 2004b, Patarnello et al. 2007).

Figure 7: Main current patterns in the area of distribution of the above mentioned species (map adapted from The Cartographic Research Lab n.d. and current patterns from Gysels et al. 2004b)

Pomatoschistus microps

Using both allozyme markers and mtDNA Gysels et al. (Gysels et al. 2004a) did a very comprehensive study on the common goby's population structure, covering its entire area of distribution. In accordance with the studies of Berrebi et al. (Berrebi et al. 2009) and contrasting with the lack of differentiation between populations of its congener *P. minutus*, the common goby has a marked population stratification with a pattern of isolation-by-distance: the Mediterranean population is clearly separated from the Atlantic populations and the latter is divided in two groups, southern and northern Atlantic, with discontinuities around the British Isles. Overall this population structure follows a consistent pattern that can be

explained by larval dispersal via oceanic and coastal currents such as the NAC and the SEC (with both FIC and ESAI playing also an important role).

In the Mediterranean *P. microps* is sedentary and its non-migratory behavior explains the differences found between populations occurring at short distances (25 km). Haplotype analyses have shown clear differences between populations from the different lagoons in the south of France. This points to a common origin with no recent gene flow between the populations, suggesting an exceptional sedentary behavior. These populations have been reported to even have different growth, fecundity and reproductive effort (Berrebi et al. 2009).

The sedentary behavior of Mediterranean common gobies coupled with the existence of the Almería-Oran Front separating the Mediterranean Sea and the Atlantic Ocean makes it very likely that gene flow between these two areas is either extremely reduced or inexistent. If existent, gene flow will be unidirectional since only populations from the Atlantic are migratory. In fact Gysels et al. (Gysels et al. 2004a) has found no common haplotypes between the population from Faro (south Portugal, NE Atlantic) and the western Mediterranean. Also the allozyme allele frequencies had pronounced differences supporting the theory that the contemporary gene flow between these two areas does not occur.

Pomatoschistus minutus

The sand goby's population structure has been studied by several authors by means of different criteria and the results obtained depend on the resolution of those criteria. Meristic characters (see for example Stefanni 2000; Webb 1980; McKay & Miller 1997), the pattern of the head sensory papillae (see for example Stefanni 2000; Webb 1980; McKay & Miller 1997), geometric morphometrics (Stefanni 2000), mt DNA and allozyme polymorphisms (see for example Stefanni et al. 2003; Stefanni & Thorley 2003; Pampoulie et al. 2004), although useful in the separation of species, have proven to be inefficient in the discrimination between populations. Among the tools used in the attempt of clarifying the population structure of the sand goby the analysis of the mtDNA has proven to be the only one yielding results, although there are still some contradicting opinions. Due to the nature of the connection between the Atlantic Ocean and the Mediterranean Sea gene flow between populations of those two locations is likely to be unidirectional (from the Atlantic ocean towards the Mediterranean sea) or even not to occur (Larmuseau et al. 2009b) and consequently although the Mediterranean populations shows some haplotypes typical from the Atlantic there is also a high level of endemic haplotypes (Gysels et al. 2004a, Gysels et

al. 2004b). Even so while some authors consider that this difference is not relevant (Stefanni et al. 2003) some others state that the degree of differentiation of the populations is significant (Miller 1986, Larmuseau et al. 2009b).

Although water exchange between the North Sea and the Baltic Sea is greatly limited studies performed up to this date have shown no relevant differences between these two locations (Stefanni & Thorley 2003). One possible explanation might be the lack of resolution of mtDNA to detect processes of contemporary and/or recent population divergence. On the contrary, and according to the study performed by Larmuseau et al. (Larmuseau et al. 2009b) using mtDNA and combining the results with previous data from other authors using other methods, the Iberian Peninsula is seemingly distinct from the northern Atlantic populations, appearing as a separate cluster in the analysis probably as a result of the existence of a glacial refugium in that location (Gysels et al. 2004a, Larmuseau et al. 2009b).

Regardless of the lack of differentiation found in some cases there is some evidence for a pattern of isolation-by-distance when geographical distance is plotted against genetic distance (Gysels et al. 2004b). In order to settle the population structure of this species, particularly to define the phylogeography of the Black Sea population, and to clarify historical processes that may be responsible for the contemporary geographic distribution of the sand goby more studies are recommended where more sensitive genetic markers (e.g. microsatellites) should be used, as well as a more comprehensive sampling scheme.

1.3 INTER- AND INTRASPECIFIC VARIATION OF OTOLITH MORPHOLOGY AND SHAPE

Fishes have in both inner ears three pairs of calcium carbonate structures, the otoliths (Figure 8), acting as mechano-electrical receptors transducing sound, acceleration and gravity. The sound frequencies to which the otolith responds to depends on its the shape (Popper & Coombs 1982, Gauldie 1988, Aguirre & Lombarte 1999, Morales-Nin 2000). These three pairs (lapilli, asteriscus and sagittae) differ in their size and shape, being the sagittae the largest and the most widely used (Tuset et al. 2003a, Monteiro et al. 2005, Ponton 2006). Otolith's morphological characteristics are considered sufficiently conserved to be regarded as either species- or genus-specific. Their shape varies over the course of growth and it is also dependent on the auditory response of the fish ear (Popper & Coombs 1982, Gauldie 1988, Lombarte & Castellón 1991, Lombarte & Lleonart 1993, Mosegaard & Morales-Nin 2000, Tuset et al. 2003a, Galley et al. 2006). It is also known that sex, age, year

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class and stock (Cardinale et al. 2004, Galley et al. 2006) are reflected on the external morphology of the otoliths. Size and shape have also been reported to be directly or indirectly influenced by environmental factors such as water temperature, diet, depth and type of substrate (Popper & Coombs 1982, L'Abee-Lund & Jensen 1993, Lombarte & Lleonart 1993, Gauldie & Crampton 2002, Tuset et al. 2003a, Tuset et al. 2003b, Volpedo & Echeverría 2003, Cardinale et al. 2004) and some authors argue that its shape is genetically determined (Lombarte & Lleonart 1993, Aguirre & Lombarte 1999, Cardinale et al. 2004).
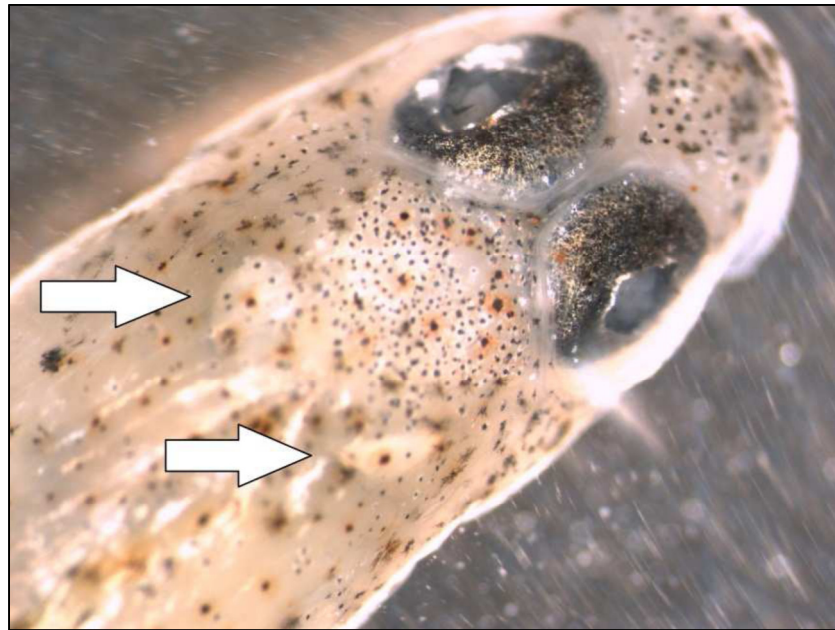
Figure 8: Location of the left and right sagittae in *Pomatoschistus minutus* (R. Guillot, 2012)

Lombarte and Lleonart (Lombarte & Lleonart 1993) have argued that genetic background determines shape while environmental conditions regulate the quantity of deposited material. Its size and shape has also been said to be altered by regional differences in fish metabolic activity which changes the growth patterns of otoliths and consequently makes otoliths good markers for stock separation. Nevertheless some caution in interpreting the results is essential since its use does not distinguish between environmental and genetic differences (Campana & Casselman 1993, Cardinale et al. 2004). The variation in morphology connected to its growth by accretion of increments and the relation between environmental conditions and chemical composition turns otoliths' shape (see for example (Campana & Casselman 1993, L'Abee-Lund & Jensen 1993, Galley et al. 2006)) and chemistry (e.g.

(Thresher et al. 1994, Swan et al. 2003, Rooker et al. 2003)) into useful tools for [1] studies of fish aging (Francis & Campana 2004), [2] past and present species identification (Tuset et al. 2003a, Hufthammer et al. 2010), [3] distinction between stocks or populations (Galley et al. 2006, Burke et al. 2008a), [4] ecomorphological studies (Arellano et al. 1995, Aguirre & Lombarte 1999), [5] migratory patterns in fossil and contemporary species (Guelinckx et al. 2008b, Geffen et al. 2011) [6] identification of prey from stomach contents (Duffy & Laurenson 1983, Doornbos 1984) and [7] the study of phylogenetic patterns (Maisey 1987, Assis 2003).

1.4 MOTIVATION AND OBJECTIVES

These three species have a very broad geographic distribution that overlaps in the European Atlantic coast (Arellano 1995). Different populations of the same species are therefore subjected to a vast range of environmental conditions while populations of different species in a given location live under the same environment. It is likely that there is a high degree of phenotypic plasticity at both the species and population level which might counteract the effects of a common genetic background. To disentangle if otoliths' shape is influenced by the environment and/or the genetic background of the populations/species, this thesis aims at:

1. identifying intra- and interspecific variability in otolith shape;
2. investigating if otolith morphology is population or species-specific;
3. investigating whether intraspecific differences in otolith morphology from geographically separated populations of *Pomatoschistus microps* and *Pomatoschistus minutus* are smaller than differences in otoliths' shape of closely related Gobiidae from the same location.
4. speculating whether the obtained results are due to environmental differences or related to the genetic background of the individuals;

The morphological measurements of otoliths and its contour will be coupled with the study of the condition of the animals and throught multivariate analysis all information will be compiled and analysed in order to reach the abovementioned goals.

II.   MATERIALS AND METHODS

2.1 FIELD SAMPLING

Animals were collected between 2009 and 2011 using a 1 m-wide beam trawl fitted with a 5 mm mesh size at the cod end and a tickler chain. The sampling locations are spread throughout the gobies' distributional range (see figure 9 and table 1 for more details). In total three species were analysed: *Pomatoschistus microps* (Pmic) (Figure 1), *Pomatoschistus minutus* (Pmin) (Figure 3) and *Pomatoschistus pictus* (Ppic) (Figure 5).

Location	Latitude	Time of collection	Pmic n	Pmin n	Ppic n	N
Bergen NO	60,45ºN	December 2009	104	74	73	251
		October 2011	33	50		83
Texel NL	53,02ºN	August 2011	14	123		137
Minho PT	41,91ºN	December 2010	21			21
		October 2010	147	19		166
		October 2009	72			72
		May 2009	109	6		115
Valosen NO	67,27ºN	September 2011	2	40		42
Trondheim NO	63,31ºN	September 2011	15	102		117
Innhavet NO	67,96ºN	September 2011		77		77
Skibotn/Sørbotn NO	69,46ºN	July 2010	32	85		117
		September 2011	6	49		55
			555	625	73	1253

Table 1: Number of specimens collected per species in each sampling location. NO: Norway; NL: The Netherlands; PT: Portugal; Pmic: *Pomatoschistus microps*; Pmin: *Pomatoschistus minutus*; Ppic: *Pomatoschistus pictus*; n: number of specimens collected; N: total number of specimens collected

Figure 9: Location of the sampling stations (adapted from The Cartographic Research Lab); 1: Bergen; 2: Texel; 3: Minho; 5: Valosen; 7: Trondheim; 8: Innhavet; 9: Skibotn/Sørbotn

2.2 OTOLITH COLLECTION

In order to avoid biases after field collection all animals were put in plastic bags and frozen for at least 2 months before registering wet weight and total length. The specimens were sexed, total length (TL) measured, wet weight (WW) registered and both sagitta were removed. Due to a very large number of females compared to males the variable "sex" was not included in the analysis. Left sagittae was weighed (Wot) and used for image analysis.

When the left sagittae was damaged during extraction or handling no picture was taken and therefore the specimen was not included in the morphology analysis. In total 965 otoliths were analysed (68 *P. pictus*, 478 *P. minutus* and 419 *P. microps*). At the beginning of each session a calibration picture was recorded. Left sagittae was photographed with sulcus side down (Figure 10) with a Nikon DS-Fi1 camera with controller DS-U2 attached to a stereo microscope, Leica MZ9.5.

Figure 10: Left sagitta from *Pomatoschistus microps* with sulcus side down (left), the overexposed left sagitta of the same individual (centre) and the binary image of the same sagitta after being processed on ImageJ (right)

The imaging software used is Nikon NIS Elements F version 2.30. The otoliths were overexposed (Figure 10) using a black background in order to obtain a high contrast and a sharp white edge of the otoliths. Using the "Batch_Set_Scale" macro all the pictures were first calibrated using the calibration image as a reference. Outlines were then extracted with ImageJ 1.45i software developed by W. Rasband at the NIH (freely available at http://rsb.info.nih.gov/ij/) (Rasband 2009) (Figure 10) and otolith area (A), otolith perimeter (Perim), circularity (Circ) and Feret's diameter (Feret) were recorded using the "Otolith_Shape_Analysis" macro. This macro defines these measurements as:

- Circularity = $4\pi(\text{area}/\text{perimeter}^2)$; Circularity values lie between 0 and 1 where 1 indicates a perfect circle and the closer this value is to 0, the more elongated the otolith is. This ratio has no units;
- Area: it is the area of the otolith. It is measured in square pixels and when properly calibrated it is shown in the desirable units (in this case mm^2);
- Perimeter: it is the length of the outside boundary of the otolith. After proper calibration it is shown in mm;
- Feret's diameter: it is the distance between the two points that are the furthest apart. It is also shown in mm after calibration;

The *bmp* images originated in the previous step were further used in SHAPE v1.3 (Iwata & Ukai 2002)software. This program extracts and decomposes the contour of the otolith in a series of orthogonal terms (the elliptic Fourier descriptors or harmonics) that are a series of sine and cosine curves (Farias et al. 2009). First the ChainCoder program included in the package extracts the contour of the otolith. Using the chain-coded information the Chc2Nef program gives the normalised EFD coefficients using a discrete Fourier transformation of those chain-codes (Figure 11). These EFDs are saved as series of an, bn, cn and dn coordinates. The an and bn are coefficients values for the elliptical Fourier expansion of the sequences to the x-coordinates while cn and dn are coefficients values of the sequences to the y-coordinates. Because these EFDs are (automatically) normalised in relation to the first harmonic they do not vary with starting point, rotation and size (Iwata and Ukai, 2002).
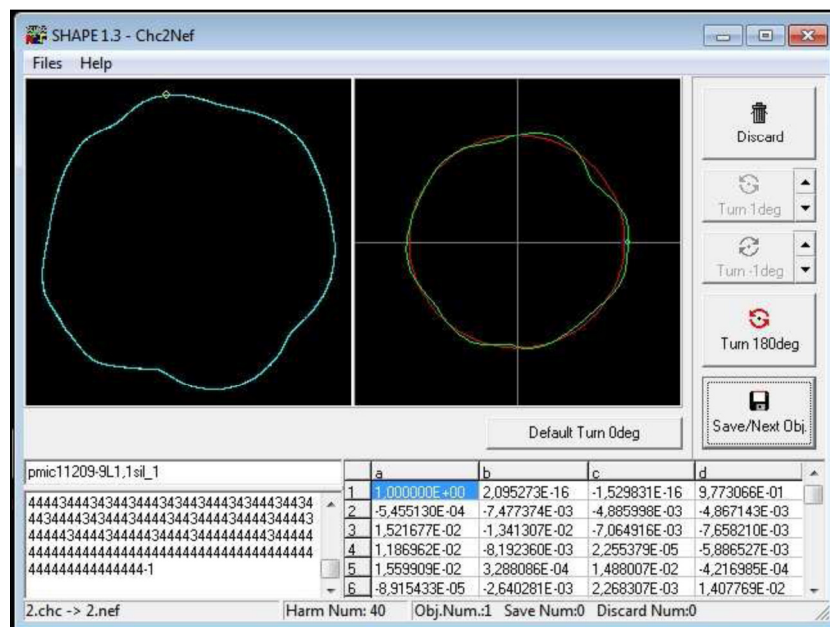
Figure 11: Results of the elliptic Fourier transformation program (from SHAPE - Iwata & Ukai 2002)

Area, perimeter, Feret's diameter and otolith weight were standardized by the total length of the fish, while circularity remained unstandardized. These measurements were combined with 15 EFDs that were chosen by previous visual inspection as representing the EFDs with the most variation (Appendix 39).

2.3 LENGTH-WEIGHT RELATIONSHIPS, RELATIVE CONDITION FACTOR AND CUMULATIVE CONDITION DISTRIBUTIONS (CCDs)

Condition factors are commonly used to compare the well-being of specimens. They are based on the assumption that heavier fishes of a certain length are in better condition that lighter fishes of that same length (Le Cren 1951, Froese 2006). The relationship between fish size and fish weight is allometric in most of the fishes, meaning that the increase in weight is bigger than the increase in length would imply (Fulton, 1904, from Froese, 2006). The allometric function generally used to describe the length-weight relashionships (LWR) is (Keys, 1928, following Froese, 2006):

$$W = {}_aL^b \text{ (1)}$$

where W is weight, L is length, and a and b are the parameters. Keys (Keys, 1928, following Froese 2006) also established its logarithm equivalent:

$$\log W = \log a + b \log L \text{ (2)}$$

where W and L are defined as above, a is a constant and b the exponent. The exponent b is generally used as b=3 and it lies within the interval 2,5<b<4 (Le Cren 1951). To compensate for changes in form or condition related with the increase in length Le Cren (Le Cren 1951) introduced the relative condition factor (K_{rel}):

$$K_{rel} = W / {}_aL^b \text{ (3)}$$

K_{rel} compares the observed weight of an individual with the mean weight of the individuals with the same length. In this case the coefficients a and b are determined empirically from the data using the linear regression on the log-transformed total length (TL in cm) and wet weight (WW in g).

The LWR of the different species/populations was expressed as:

$$\log WW = a^1 + b^1 \log TL \text{ (4)}$$

where WW is the wet weight, TL is the total length of the fish, a^1 is the intercept and b^1 is the slope. In order to calculate the K_{rel} equation 3 was rewritten as:

$$K_{rel} = W_{obs} / W_{est} \text{ (5)}$$

where K_{obs} represents the measured wet weight of the animal and W_{est} is the weight estimated using the length. In average K_{rel} is 1 due to the empirically estimated parameters *a* and *b* which means that if $K_{rel} < 1$ the specimen's condition is below the average and if $K_{rel} > 1$ the specimen's condition lies above the average. Mean condition of the fishes is known to vary between seasons, locations and years (Safran 1992, Andreu-Soler et al. 2006, Froese 2006). The cumulative condition distributions represent a useful tool for the comparison of the relative condition factors between the different species or populations. Since the K_{rel} of the different groups can be expressed with small overlap in the same plot the visual interpretation of the results is easier. In order to compare the K_{rel} between populations and seasons within one species and between species K_{rel} was also expressed as a CCD.

2.4 STATISTICAL ANALYSIS

All data analysis was performed using PAST software (Hammer et al. 2001) with the significance level set to α=0,05 except if stated otherwise. Outliers were identified as the data points lying three standard deviations from the mean (outlier = average ± 3*standard deviation) and were removed. Following the central limit theorem the means of the variables were considered normally distributed. Total length was used to correct the otolith measurements and it was excluded from the analyses together with wet weight in order to exclude the effect of possible differences of fish size between groups.

To test for differences in the CCDs a Kruskall-Wallis test was performed. The categories were species (*P. microps*, *P. minutus* and *P. pictus*), locations (Bergen, Texel, Minho, Trondheim and Skibotn/Sørbotn for *P. microps* and Bergen, Texel, Minho, Trondheim, Valosen, Innhavet and Skibotn/Sørbotn for *P. minutus*) and season (the four seasons compared within *P. microps* and due to a small number of individuals collected during the spring only summer, autumn and winter were compared for *P. minutus*). Two approaches were used in the interspecific comparisons: the locations were pulled together and analyzed as a whole and as a separate analysis only the population from Bergen was used. These two comparisons were made in order to understand if pulling together all the locations had any effect on the outcome. The population from Bergen was chosen because it was the only location where the three species were present.

The differences between the regressions of log TL-log A, log A-log Perim, TL-Circ, TL-Feret and TL-Wot were tested using an analysis of covariance (ANCOVA). The ANCOVA checked for differences between the same groups referred in the previous paragraph.

The differences in location, species and season were tested by a one-way multivariate analysis of variance (MANOVA) where the normalized measurements of the otoliths were coupled with the 15 EFDs. MANOVA is a generalized form of ANOVA used for comparing multivariate means of several groups. This test is more effective when the variables have some degree of correlation. When the MANOVA showed significant results between the groups a post-hoc analysis was performed by pairwise comparisons using a Hotelling's T^2 test. A canonical variate analysis (CVA) (multigroup discriminant analysis) followed the MANOVA. In a CVA the original variables are combined in a linear way in order to maximize the relative variation between groups in relation to the within-group variation. The coefficients of this linear combination give the first canonical vector. This maximizes the ratio of the between- to within-group variation (termed canonical root) resulting in the canonical variates. The CVA produces a scatterplot of the specimens along the two first canonical roots that represent the maximal separation between groups (with the canonical variates as the coordinates). The variation explained by each axis is indicated by the corresponding eigenvalues and the interpretation of the scatterplot can be done using the Phytagorean distances (Campbell & Atchley 1981, Iwata & Ukai 2002). In this analysis the degree and direction of the between- and within-group variation is determined by the degree of correlation of the variables used: when there is a high within-group correlation and low between-group correlation the CVA provides maximum discrimination (Lubischew 1962, Campbell & Atchley 1981); the opposite is also true.

RESULTS

The number of females identified was much higher than the number of males. In order to avoid biases due to differences between sexes this variable was left out of the analysis.

3.1 LENGTH-WEIGHT RELATIONSHIPS, RELATIVE CONDITION FACTOR AND CUMULATIVE CONDITION DISTRIBUTIONS (CCDs)

Interspecific comparisons

a. All populations merged

The relationship between the log of the total length and the log of the wet weight of the fish was determined for the three species (Figure 12). The parameters a^1 (intercept) and b^1 (slope), p-values and r^2 are in Table 2. All the regressions were found to be significant with *P. minutus* showing the highest correlation between the two variables (r^2= 0,86192) and *P. microps* the lowest (r^2=0,77576). The CCDs of the common and the painted goby were revealed to be quite similar (Figure 13).
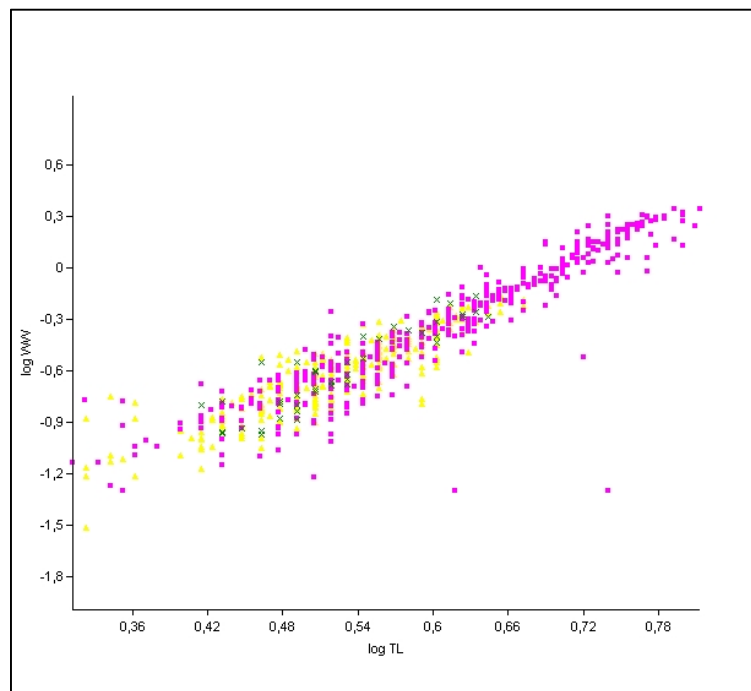
Figure 12: Length-weight relationships for *P. microps* (▲), *P. pictus* (x) and *P. minutus* (■)

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The sand goby showed a significantly higher relative condition factor (Table 3, Appendix 1). *P. microps* had the lowest average K_{rel} (1,64±1,39) while *P. minutus* showed by far the highest condition factor (10,08±13,22) but also the largest variation (Table 3). Average condition factors and Kruskal-Wallis test results are presented in Table 3 and Appendix 1, respectively.

Species	Slope	Intercept	r^2	p-value
P. microps	3,3072	-2,3502	0,77576	2,04E-112
P. minutus	3,555	-2,4985	0,86192	2,78E-191
P. pictus	3,8199	-2,6133	0,84571	4,64E-19

Table 2: Slope, intercept, r^2 and p-values of the LWRs in *P. microps*, *P. minutus* and *P. pictus*
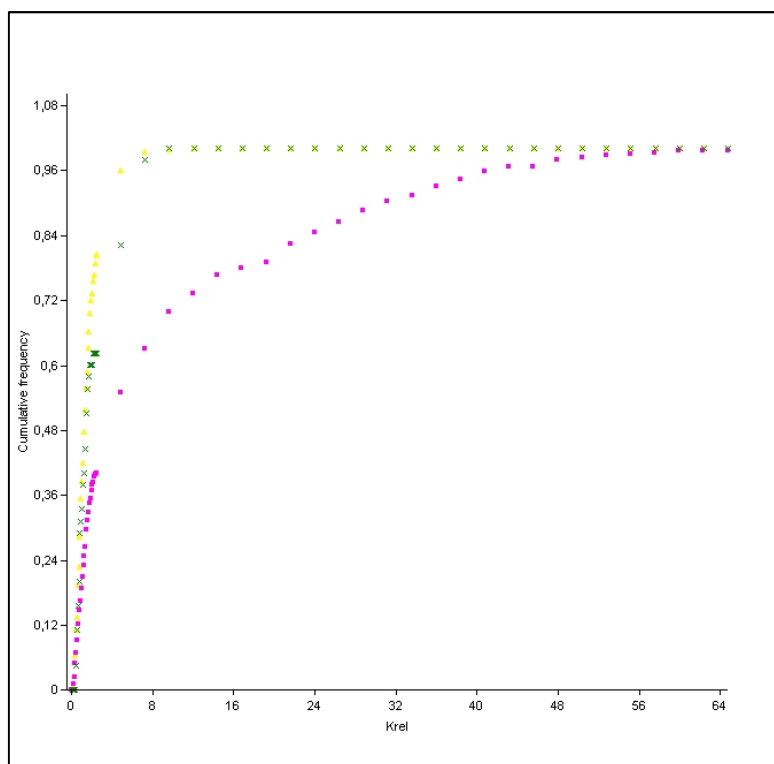
Figure 13: CCDs of *P. microps* (▲), *P. pictus* (×) and *P. minutus* (■)

	TL			WW			K_rel		
Species	**n**	**Avg (mm)**	**SD**	**n**	**Avg (g)**	**SD**	**n**	**Avg (mm)**	**SD**
P. microps	342	33,66	5,2	342	0,262	0,13	342	1,64	1,39
P. minutus	457	41,4	10,14	457	0,624	0,53	457	10,08	13,22
P. pictus	45	34,3	5,03	45	0,302	0,17	45	2,48	2,21

Table 3: Number (n), average (Avg), standard deviation (SD) and relative condition factor (K_rel) of the species used in the LWRs and CCDs

b. Bergen

The relationship between the log of the total length and the log of the wet weight of the fish was determined for the three species (Figure 14).
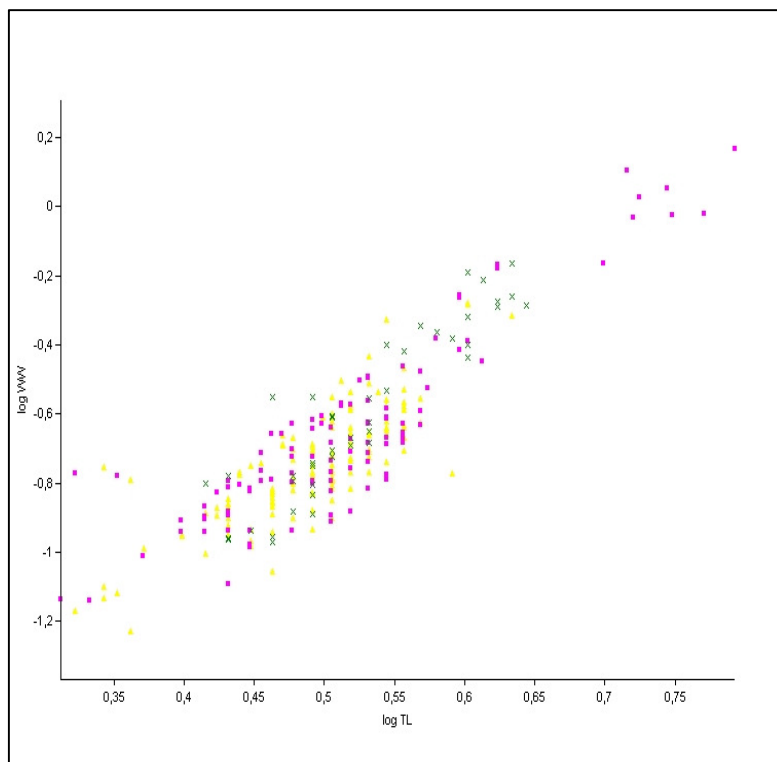
Figure 14: Length-weight relationships for the specimens caught in Bergen. *P. microps* (▲), *P. pictus* (x) and *P. minutus* (■)

The parameters a^1 (intercept) and b^1 (slope), p-values and r^2 are on Table 4. All the regressions were found to be significant with *P. pictus* showing the highest correlation between the two variables ($r^2 = 0,84589$) and *P. microps* the lowest ($r^2 = 0,63902$). The CCDs of the common and the painted goby revealed to be quite parallel and therefore similar (Figure 15).

Population	Slope	Intercept	r^2	p-value
P. microps	2,9266	-2,184	0,63902	4,19E-30
P. minutus	2,9356	-2,1645	0,81604	6,46E-39
P. pictus	3,8186	-2,6125	0,84589	4,52E-19

Table 4: Slope, intercept, r^2 and p-values of the LWRs in *P. microps*, *P. minutus* and *P. pictus* caught in Bergen

There was no significant difference between the CCDs of the three species (p=0,2305) (Appendix 2). *P. microps* had the lowest average K_{rel} (0,89±0,61) while *P. pictus* had the highest condition factor (2,48±2,21) (Table 5). Average condition values and Kruskal-Wallis test results are presented on Table 5 and Appendix 2, respectively.

Population	TL			WW			K_{rel}		
	n	Avg (mm)	SD	n	Avg (g)	SD	n	Avg	SD
P. microps	130	31,29	3,94	130	0,194	0,08	130	0,89	0,61
P. minutus	103	33,48	7,75	103	0,281	0,26	103	2,11	4,04
P. pictus	45	34,29	5,12	45	0,302	0,17	45	2,48	2,21

Table 5: Number (n), average (Avg), standard deviation (SD) and relative condition factor (K_{rel}) of the species used in the LWRs and CCDs for Bergen
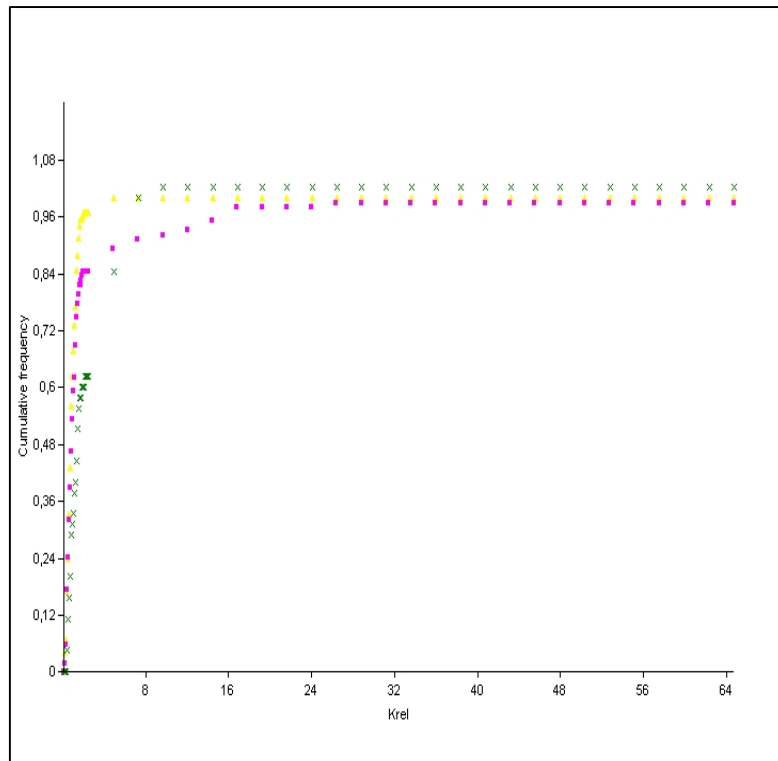
Figura 15: CCDs of *P. microps* (▲), *P. pictus* (x) and *P. minutus* (■) from Bergen

Intraspecific comparisons

a. Pomatoschistus microps

a1. Comparisons between populations

The length-weight relationships for the different populations of the common goby are shown in Figure 16 and slope, intercept, r^2 and p-values are on Table 6. All relationships were significant with the population from Skibotn/Sørbotn showing the highest correlation (r^2=0,96819) and the population from Bergen showing the lowest correlation (r^2=0,61439) between these two variables (Table 6). The CCDs show some differences between the populations (Figure 17). The Kruskal-Wallis test showed significant differences between the populations (p=1,08^{-44}) with the Post-hoc pairwise test showing differences between all pairs compared (Appendix 3). The highest K_{rel} was found in the population from Skibotn/Sørbotn (3,61±2,15) and the lowest in Bergen (0,76±0,64). Average condition values and Kruskal-Wallis test results are presented on Table 7 and Appendix 3, respectively.
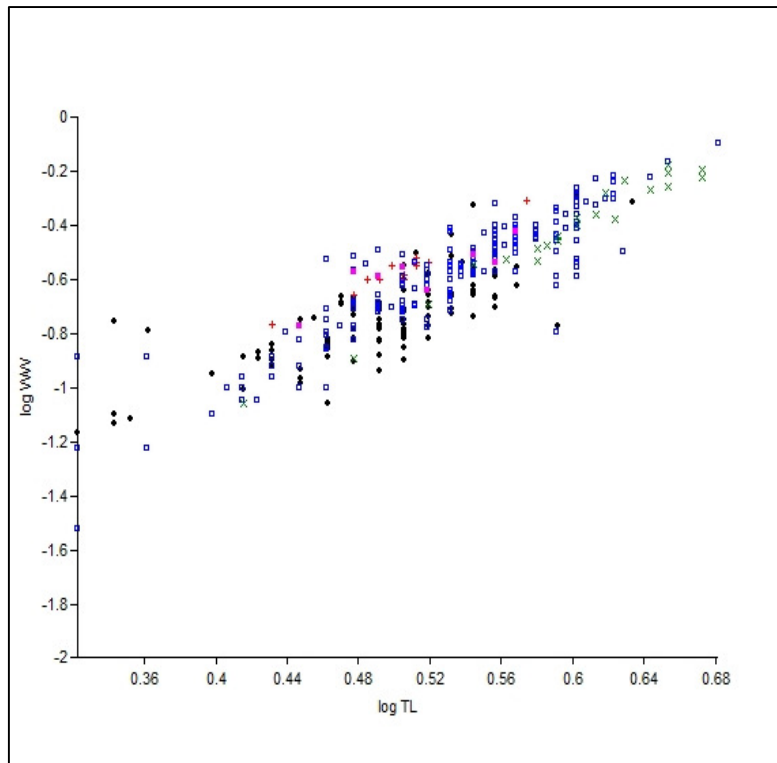
Figure 16: Length-weight relationships for *P. microps*. The populations are: Bergen (●),
Texel (+), Minho (□), Trondheim (■) and Skibotn/Sørbotn (x)

Population	Slope	Intercept	r²	p-value
Bergen	2,9019	-2,1737	0,61439	4,83E-23
Texel	3,249	-2,2003	0,95105	1,49E-08
Minho	3,3801	-2,381	0,79514	2,51E-67
Trondheim	3,6214	-2,4452	0,77464	0,00174
Skibotn/Sørbotn	3,5586	-2,5457	0,96819	1,90E-16

Table 6: Slope, intercept, r² and p-values of the LWRs in the studied populations of *P.
microps*
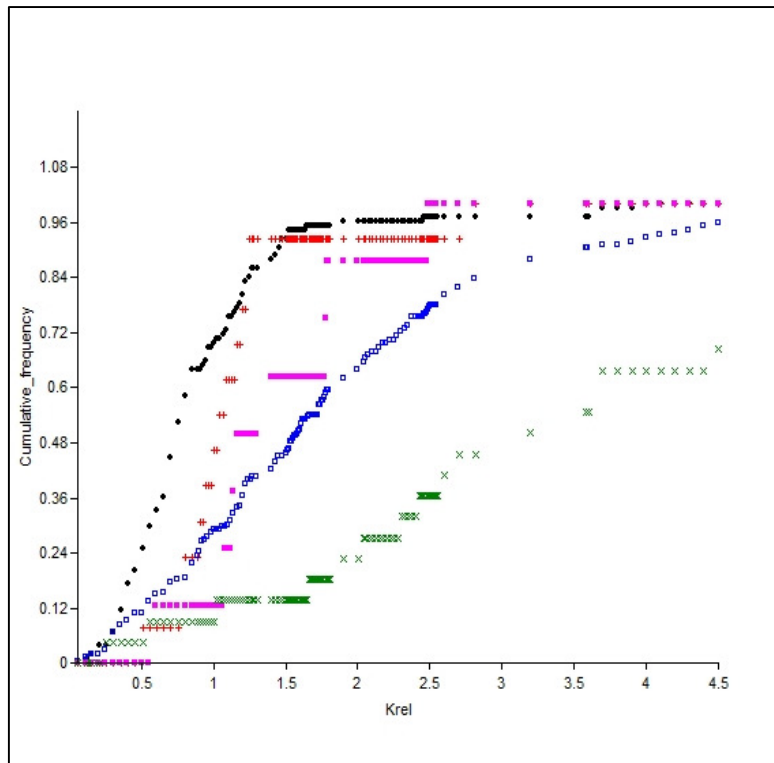
Figure 17: CCDs of *P. microps*: Bergen (●), Texel (+), Minho (□), Trondheim (■) and Skibotn/Sørbotn (x)

Population	Slope	Intercept	r²	p-value
Bergen	2,9019	-2,1737	0,61439	4,83E-23
Texel	3,249	-2,2003	0,95105	1,49E-08
Minho	3,3801	-2,381	0,79514	2,51E-67
Trondheim	3,6214	-2,4452	0,77464	0,00174
Skibotn/Sørbotn	3,5586	-2,5457	0,96819	1,90E-16

Table 7: Number (n), average (Avg), standard deviation (SD) and relative condition factor (K$_{rel}$) of the populations of the common goby used in the LWRs and CCDs

a2. Comparison between seasons

The length-weight relationships for the different seasons of the common goby are shown in Figure 18 and slope, intercept, r² and p-values are in Table 6. All relationships were significant with the summer season showing the highest correlation (0,85662) and the winter months showing the lowest (0,63876) (Table 8). The CCDs are relatively similar (Figure 19)

with the summer months showing the highest K$_{rel}$ (2,13±1,47) and the winter months showing the lowest (1,12±0,67) (Table 9). The Kruskal-Wallis test results showed significant differences between the seasons (p=1,66E-20) with the Post-hoc pairwise tests also showing significant differences between the seasons (Appendix 4). Average condition values and Kruskal-Wallis test results are presented on Table 9 and Appendix 4, respectively.

Figure 18: Length-weight relationships for the different seasons of *P. microps*' populations. Spring (x), summer (▲), autumn (ı) and winter (●)

Season	Slope	Intercept	r^2	p-value
Spring	3,2257	-2,276	0,6933	1,14E-27
Summer	2,6273	-1,9504	0,85662	1,30E-17
Autumn	3,2679	-2,3297	0,82546	5,51E-77
Winter	3,4646	-2,4899	0,63876	3,59E-23

Table 8: Slope, intercept, r^2 and p-values of the LWRs in the studied seasons of *P. microps*
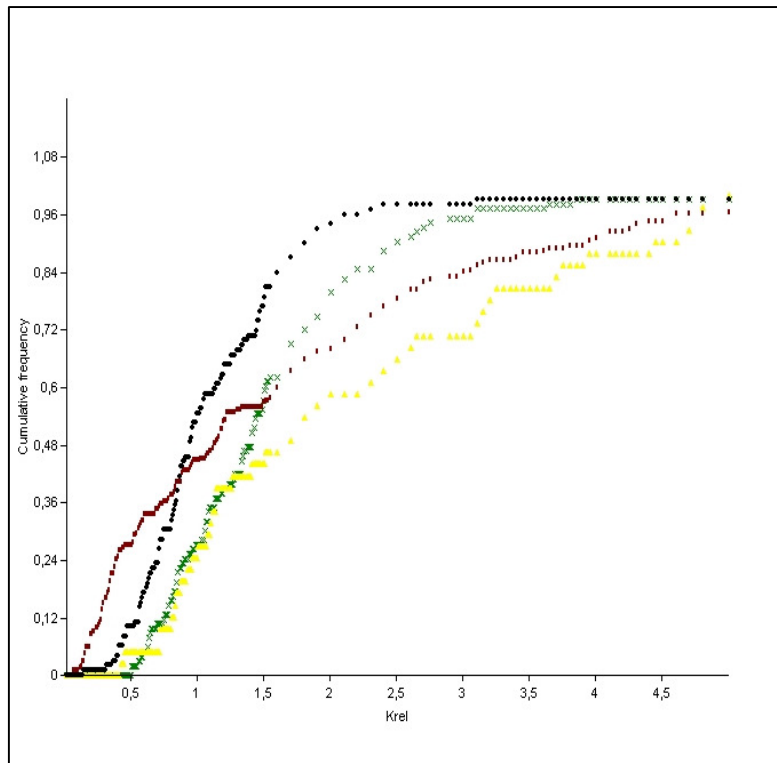
Figure 19: CCDs of *P. microps*' populations caught in the different seasons: spring (x), summer (▲), autumn (ı) and winter (●)

Season	**TL**			**WW**			**K_{rel}**		
	n	**Avg (mm)**	**SD**	**n**	**Avg (g)**	**SD**	**n**	**Avg**	**SD**
Spring	107	34,09	3,46	107	0,283	0,1	107	1,56	1,01
Summer	45	38	6,02	45	0,377	0,15	45	2,13	1,37
Autumn	265	33,32	6,2	265	0,254	0,15	265	1,59	1,42
Winter	99	32,25	3,06	99	0,193	0,07	99	1,12	0,67

Table 9: Number (n), average (Avg), standard deviation (SD) and relative condition factor (K_{rel}) of the different seasons of the common goby used in the LWRs and CCDs

b. Pomatoschistus minutus

b1. Comparisons between populations

The length-weight relationships for the different populations of the sand goby are shown in Figure 20 and slope, intercept, r^2 and p-values are on Table 10.

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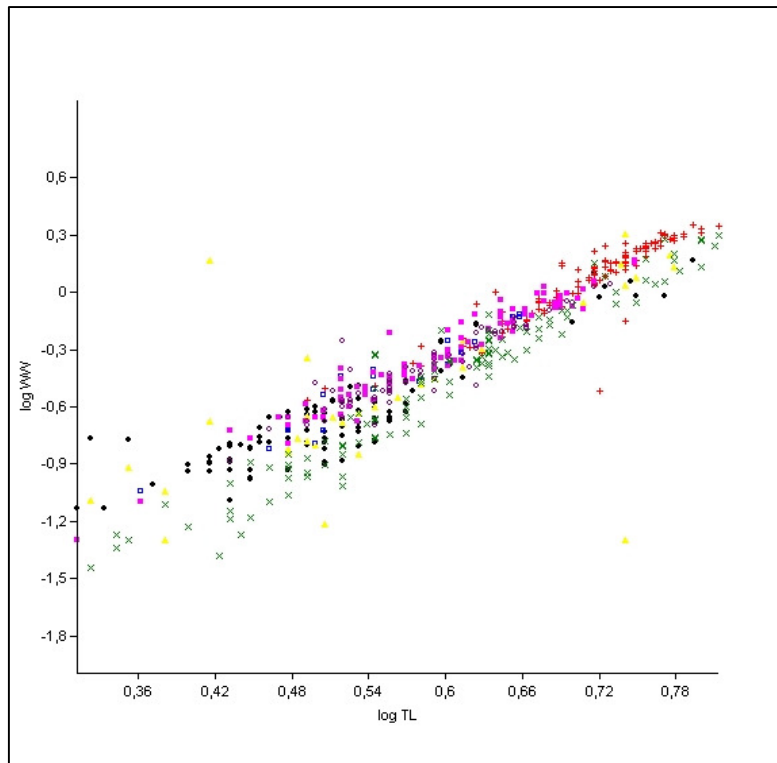
Figure 20: Length-weight relationships for *P. minutus*. The populations are: Bergen
(●), Texel (+), Minho (□), Valosen (▲), Trondheim (■) , Innhavet (○) and Skibotn/Sørbotn (x)

Population	Slope	Intercept	r^2	p-value
Bergen	2,928	-2,1614	0,81552	1,75E-38
Texel	3,4691	-2,4032	0,8476	8,47E-49
Minho	3,2776	-2,2948	0,92299	2,68E-14
Valosen	3,5	-2,4788	0,45635	1,61E-05
Trondheim	3,2257	-2,2458	0,94535	2,12E-62
Innhavet	3,0881	-2,201	0,87302	1,92E-34
Skibotn/Sørbotn	3,7977	-2,7431	0,93978	3,12E-73

Table 10: Slope, intercept, r^2 and p-values of the LWRs in the studied populations of *P. minutus*

All relationships were found to be significant with the population from Skibotn/Sørbotn showing the highest correlation (r^2=0,93978) and the population from Valosen showing the lowest correlation (r^2=0,45635) between these two variables (Table 10). The CCDs of the

different populations are relatively parallel with the exception of the population from Texel that showed by far the highest average condition among the populations sampled (21,27±12,92) (Table 11, Figure 21). The Kruskal-Wallis test showed significant differences between the populations (p=3,81E-70) and the Post-hoc pairwise test showed differences between all pairs compared (Appendix 5). Average condition values and Kruskal-Wallis test results are presented on Table 11 and Appendix 5, respectively.
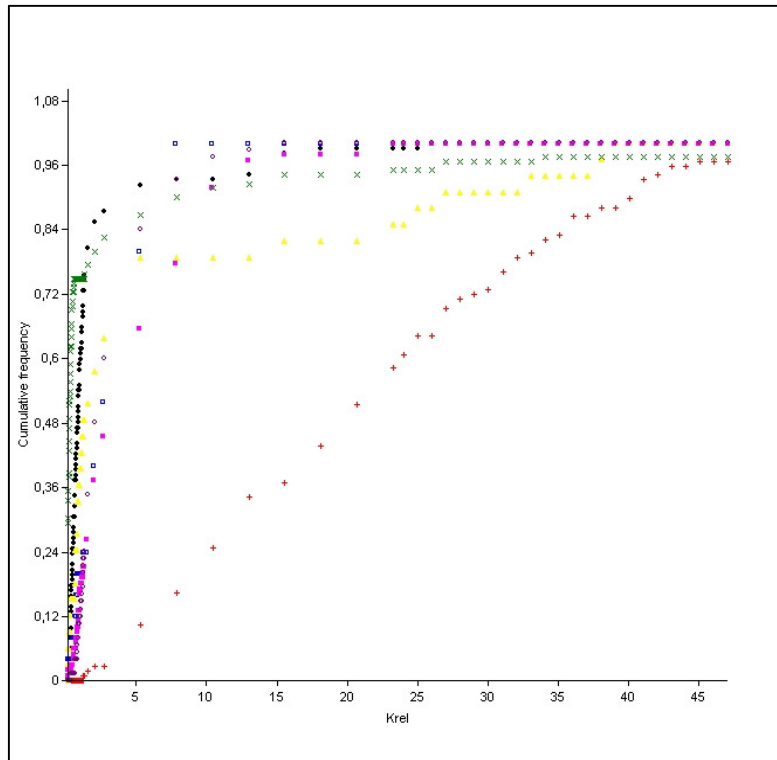
Figure 21: CCDs of *P. minutus*: Bergen (●), Texel (+), Minho (□), Valosen (▲), Trondheim (■) , Innhavet (○) and Skibotn/Sørbotn (x)

	TL			WW			K$_{rel}$		
Population	n	Avg (mm)	SD	n	Avg (mm)	SD	n	Avg	SD
Bergen	102	33,43	7,78	102	0,27	0,24	102	2,087	4,04
Texel	117	52,21	7	117	1,257	0,5	117	21,27	12,92
Minho	25	37,54	5,94	25	0,422	0,2	25	3,176	0,24
Valosen	33	37,06	11,6	33	0,499	0,53	33	7,164	11,64
Trondheim	99	39,7	7,21	99	0,537	0,3	99	4,65	4,24
Innhavet	75	38,01	5,78	75	0,424	0,21	75	3,118	2,74
Skibotn+Sørbotn	119	40,67	9,91	119	0,493	0,45	119	3,79	12,17

Table 11: Number (n), average (Avg), standard deviation (SD) and relative condition factor (K$_{rel}$) of the different populations of the sand goby used in the LWRs and CCDs

b2. Comparison between seasons

The length-weight relationships for the different seasons of the sand goby are shown in Figure 22 and slope, intercept, r^2 and p-values are on Table 12. Due to a small number of individuals caught during spring (Table 13) this season was excluded from the remaining analysis. All relationships were significant with the winter season showing the highest correlation (r^2=0,89807) and the autumn months showing the lowest (r^2=0,75834) (Table 12).

Season	Slope	Intercept	r^2	p-value
Spring	Too few individuals			
Summer	3,7361	-2,6235	0,86126	2,83E-81
Autumn	3,5277	-2,4676	0,75834	2,53E-101
Winter	3,3139	-2,447	0,89807	6,03E-27

Table 12: Slope, intercept, r^2 and p-values of the LWRs in the studied seasons of *P. minutus*

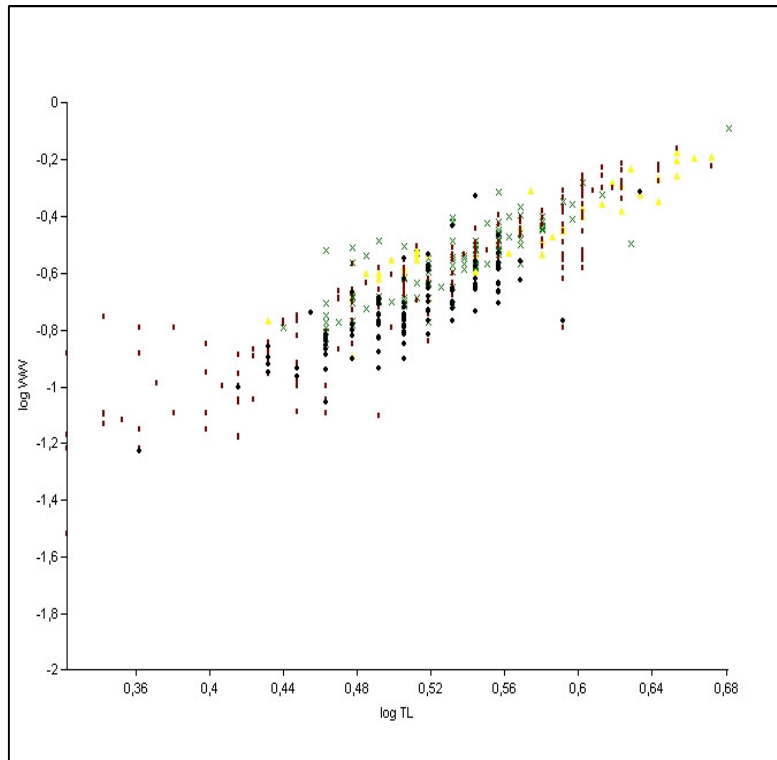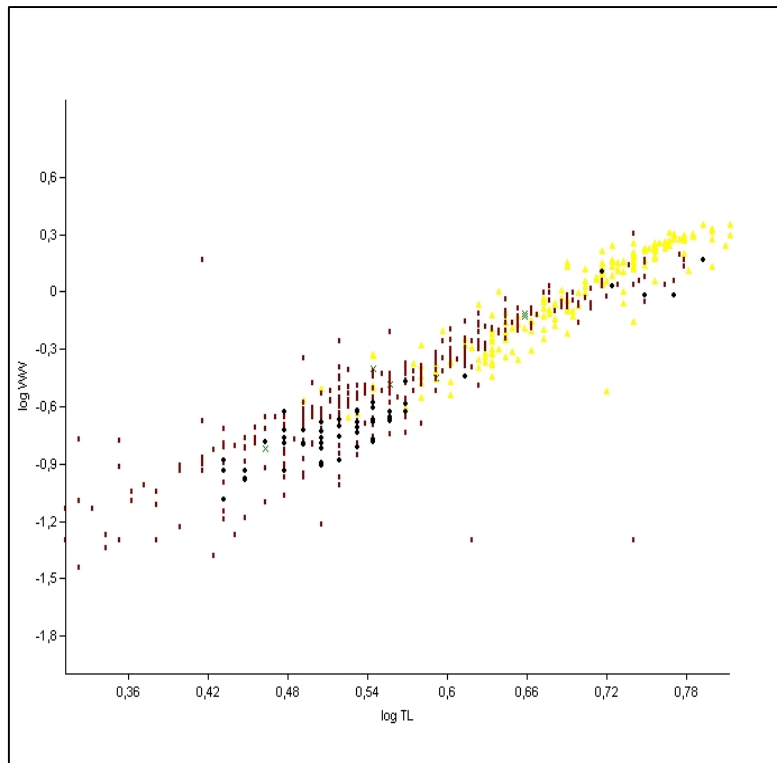**Figure 22**: Length-weight relationships for the different seasons of *P. minutus*' populations. Spring (x), summer (▲), autumn (ı) and winter (●)

The CCDs of the winter and autumn months are relatively parallel (Figure 23) with the summer months showing highest higher K_{rel} (23,41±18,82) and the winter months showing the lowest (3,97±7,17) (Table 13).

The Kruskal-Wallis test results showed significant differences between the seasons (p=1,24E-112) with the Post-hoc pairwise tests also showing significant differences between the seasons (Appendix 6). Average condition values and Kruskal-Wallis test results are presented on Table 13 and Appendix 6, respectively.
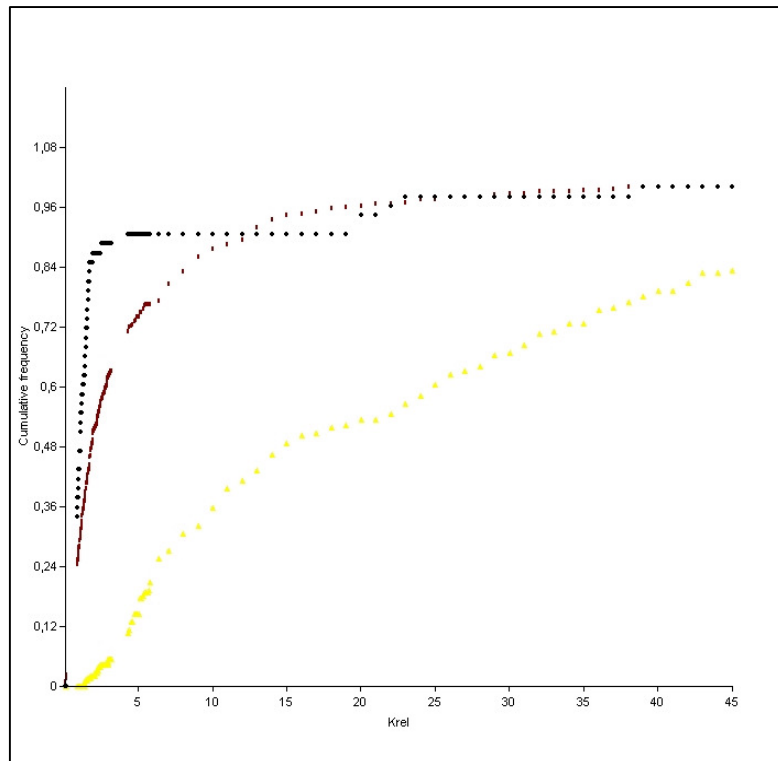
Figure 23: CCDs of *P. minutus*' populations caught in the different seasons: summer (▲),
autumn (I) and winter (●)

	TL			WW			K_{rel}		
Season	n	Average (mm)	SD	n	Average (mm)	SD	n	Average	SD
Summer	187	49,4	7,68	46	1,031	0,53	45	23,41	18,82
Autumn	324	36,82	8,23	273	0,468	0,33	265	5,49	7,79
Winter	48	35,52	7,86	104	0,334	0,32	99	3,97	7,17

Table 13: Number (n), average (Avg), standard deviation (SD) and relative condition
factor (K_{rel}) of the different seasons of the sand goby used in the LWRs (with the
exception of the spring months) and CCDs

3.2 OTOLITH MORPHOLOGICAL PATTERNS AND ITS RELATIONSHIP WITH OTOLITH SIZE

Interspecific comparisons

a. All populations merged

Area, perimeter, Feret's diameter and otolith weight were standardized for total length. Circularity, as a ratio, remained unstandardized. The mean, standard deviation and range of the morphological variables are shown on Appendix 7. The linear regressions between log TL-log A, log A-log Perim, TL-Circ, TL-Feret and TL-Wot for the three species are shown on Appendix 8. The ANCOVA results for the slopes and intercepts are shown on Appendix 9. All linear regressions for the 3 species were significant with the results from the ANCOVA also showing significant differences in all the comparisons.

As expected the three species showed the same tendency in all the comparisons: the bigger the total length, the bigger the area, Feret's diameter and otolith weight. The same tendency was found in the linear regression between the logarithm of the area of the otolith and the logarithm of its perimeter: the bigger the area, the bigger the perimeter. Contrarily to the previous relationships, when the animal was bigger the otolith was less circular. Also when the animals were bigger there was a higher range in the otolith's circularity (Appendix 10). In general *P. pictus*' and *P. microps*' otoliths have approximately proportional relationships between the variables while *P. minutus*' otoliths show a considerable difference: proportionally otoliths from the sand goby not only tend to be heavier in larger specimens but also larger and less circular than otoliths from the common and the painted goby. The sand goby also shows higher values of Feret diameter. At all sizes when the painted goby is compared with the common goby it shows proportionally smaller otoliths which are more circular (Appendix 14).

b. Bergen

The mean, standard deviation and range of the morphological variables are shown on Appendix 11. The linear regressions between log TL-log A, log A-log Perim, TL-Circ, TL-Feret and TL-Wot for the three species are shown on Appendix 12. The ANCOVA results for the slopes and intercepts are shown on Appendix 13. All linear regressions for the 3 species were significant. All intercepts were statistically significant, with the exception of the

regression between the logarithm of the area and the logarithm of the perimeter (p=0,2591). There were significant differences between all the slopes compared (Appendix 13).

P. pictus' otoliths tend to have a proportionally smaller area and Feret's diameter when compared with *P. microps* and *P. minutus*. Also *P. microps*' otoliths tend to be more circular and less heavy than the otoliths from the other 2 species.

Intraspecific comparisons

a. *Pomatoschistus microps*

a1. Comparisons between populations

The linear regressions between log TL-log A, log A-log Perim, TL-Circ, TL-Feret and TL-Wot for the different populations of the common goby are shown in Appendix 15. All regressions were significant with the exception of the regression between TL and circularity for the populations of Texel (p=0,10756) and Trondheim (p=0,13419) (Appendix 15 and 17). The ANCOVA Tables for the different comparisons are found on Appendix 16. The ANCOVA showed significant differences for all the relationships in the slopes and intercepts between the different populations, with the exception of the regression between TL and circularity that showed homogeneity of slopes (p=0,05385) (Appendix 16).

All populations showed the same trend in all the comparisons: the bigger the total length, the bigger the area, Feret's diameter and otolith weight. The same tendency was found in the linear regression between the logarithm of the area of the otolith and the logarithm of its perimeter: the bigger the area, the bigger the perimeter. Contrarily to the previous relationships, when the animal was bigger the otolith was less circular. Also when the animals were bigger there was a higher range in the otolith's circularity (Appendix 23).

a2. Comparison between seasons

Appendix 18 shows the parameters a^1 and b^1, r^2 and p-values for the linear relationships between TL and Wot. All the relationships were significant. The highest correlation was found in the summer (r^2=0,86922) and the lowest in the winter (r^2=0,62037) (Appendix 18). The tendency was similar in all the seasons: the bigger the specimen, the heavier the otoliths (Appendix 20). There is a significant difference between the slopes and

intercepts between the different seasons. The ANCOVA Table for the comparison between seasons is shown on Appendix 19.

Winter and autumn showed a parallel tendency in this relationship. At any given length otoliths from fish caught in the winter were heavier than otoliths from fish caught during autumn. Spring and summer also showed a parallel regression line with otoliths from fish caught in the spring heavier than otoliths from fish caught during the summer, at any given length. The weight of the otolith becomes proportionally bigger in larger individuals caught in the spring and summer than during autumn and winter (Appendix 17).

b. *Pomatoschistus minutus*

b1. Comparisons between populations

The linear regressions between log TL-log A, log A-log Perim, TL-Circ, TL-Feret and TL-Wot for the different populations of the sand goby are shown on Appendix 21 and on Appendix 23. All regressions were found to be significant (Appendix 21). The ANCOVA Tables for the different comparisons are found on Appendix 22. All the populations showed similar tendencies in the different relationships between the variables (Appendix 23). The ANCOVA showed significant differences for all the relationships in the slopes and intercepts between the different populations (Appendix 22).

With variations in the slope and intercept all populations showed the same trend in all the comparisons: the bigger animal, the bigger the area, Feret's diameter and weight of the otolith. The same tendency was found for the linear regression between the logarithm of the area of the otolith and the logarithm of its perimeter: the bigger the area, the bigger the perimeter. When the animal was bigger the otolith was less circular. Also when the animals were bigger there was a higher range in the otolith's circularity (Appendix 23).

b2. Comparison between seasons

Due to a small number of individuals caught during the spring (5) comparisons were only made using summer, autumn and winter. Appendix 24 shows the slope, intercept, r^2 and p-values of the linear relationship between TL and Wot. All relationships were found to be significant. The summer season shows the highest correlation (r^2=0,77147) while the autumn

season shows the lowest correlation (r^2=0,59663) (Appendix 24). Appendix 25 shows the ANCOVA results for the comparisons between seasons. Both slope and intercept showed significant results (Appendix 25). All the seasons showed the same tendency, with larger animals having the heavier otoliths (Appendix 26).

Winter and autumn showed relatively parallel trends: increase in weight of the otoliths was slower than the increase in length. At any given length the weight of the otoliths from the fish caught during the winter was bigger than those of the autumn caught animals. The otoliths from the animals caught during the summer had proportionally heavier otoliths in larger individuals (Appendix 26).

3.3 OTOLITH SHAPE ANALYSIS FOR INTER- AND INTRA-SPECIFIC DIFFERENCES

3.1 Interspecific comparisons

a. All populations merged

A MANOVA was conducted in order to test the hypothesis that there would be mean differences in the otolith shape between the three species, once the EFDs and the morphological measurements were combined. The effect was found to be statistically significant: Pillais' Trace=0,4316; F= 11,23; p=3,796E-61. Since the MANOVA showed overall significant differences between the species a series of pairwise Hotelling's T^2 tests followed. All comparisons revealed statistically significant differences (Appendix 27). The maximum separation of the species produced in the CVA scatterplot can be seen on Figure 24. Due to negative scores of *P. minutus* on the second canonical root (mainly associated with Wot and V7) and positive scores of *P. microps* and *P. pictus* on that same canonical root (associated with Feret's diameter, perimeter, circularity, V17 and V9) these species are marginally separated in two groups. *P. microps* and *P. pictus* also show some degree of separation due to more positive scores on the canonical root 1 (mainly associated with perimeter, area, Variable 16 and Feret's diameter) of *P. microps* and negative scores on that same axis of *P. pictus* (mainly associated with circularity and variable 6) (Appendix 28, Figure 24).
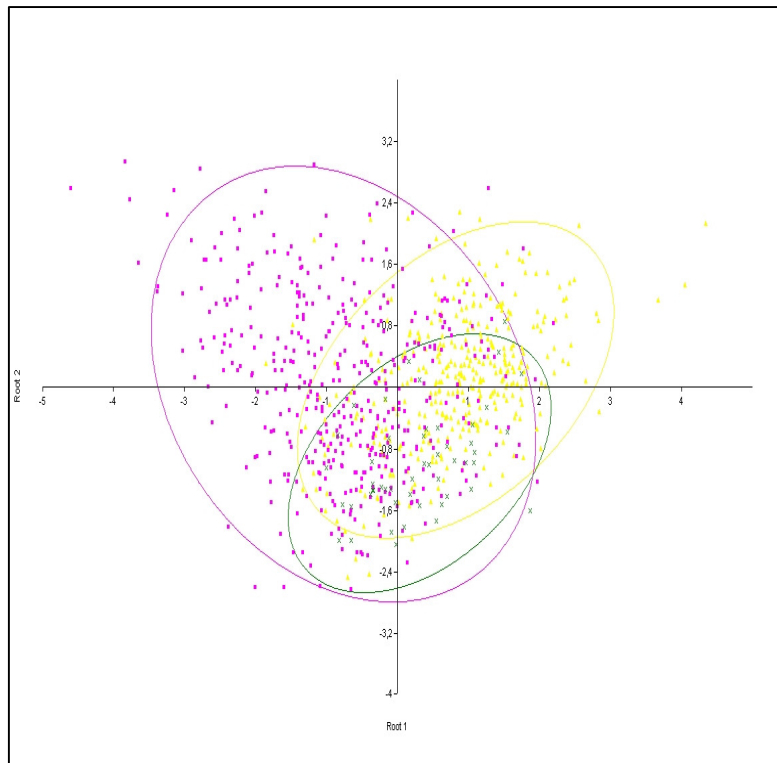
Figure 24: CVA scatterplot of the morphological variables and EFDs for *P. microps* (▲), *P. pictus* (x) and *P. minutus* (■) fitted with 95% confidence ellipses

b. Bergen

The MANOVA was found to be statistically significant: Pillais' Trace=0,4316; F= 11,23; p=3,796E-61. Since the MANOVA showed overall significant differences between the species a series of pairwise Hotelling's T^2 tests followed. All comparisons revealed statistically significant differences (Appendix 29). The maximum separation of the species produced in the CVA scatterplot can be seen on Figure 25.
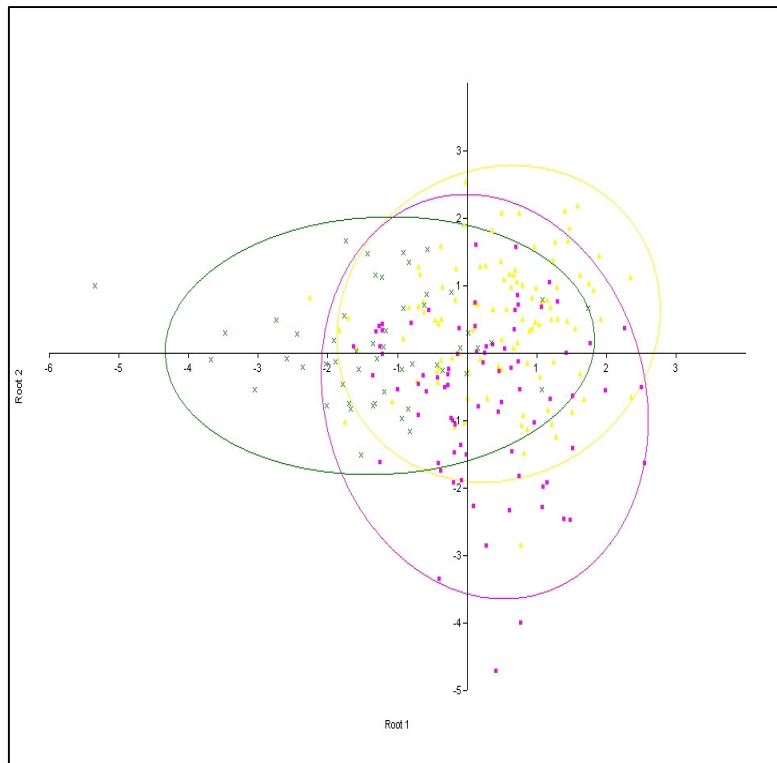
Figure 25: CVA scatterplot of the morphological variables and EFDs for *P. microps* (▲),
P. pictus (x) and *P. minutus* (■) caught in Bergen fitted with 95% confidence ellipses

There is some separation of *P. pictus*' specimens due to negative scores on root 1 which is mainly related with Variables 7, 17, 14, 24, 20, 22, 6, 13, 15 and circularity (Appendix 30). *P. microps* and *P. minutus* are overlapping although *P. microps*' specimens have a higher score on the canonical root 2 (which is associated with area, perimeter, Feret's diameter, otolith weight and variable 16) (Appendix 30, Figure 25).

Intraspecific comparisons

a. *Pomatoschistus microps*

a1. Comparisons among locations

In the comparison between locations the overall effect was found to be statistically significant: Pillais' Trace=1,155; F= 6,148; p=1,396E-48. All pairwise comparisons revealed statistically significant. The Table with the Hotelling's T^2 tests is found on Appendix 31. The

CVA scatterplot (Figure 26) revealed an almost complete separation of the northernmost population (Skibotn/Sørbotn, associated with variable 24) due to the combination of positive values on the canonical root 1 and negative values on the canonical root 2. The specimens from Bergen (associated with otolith weight, variable 6 and circularity) can also be marginally separated from the specimens from Minho (associated with variables 8, 9 13, 17, 20 and 24) due to a combination of lower scores on both axis of the first population. The specimens from Trondheim are scattered throughout the plot overlapping with the other populations while the specimens from Texel can only be found on the positive side of the canonical root 2, overlapping with the population from Minho and Trondheim (Appendix 32, Figure 26).
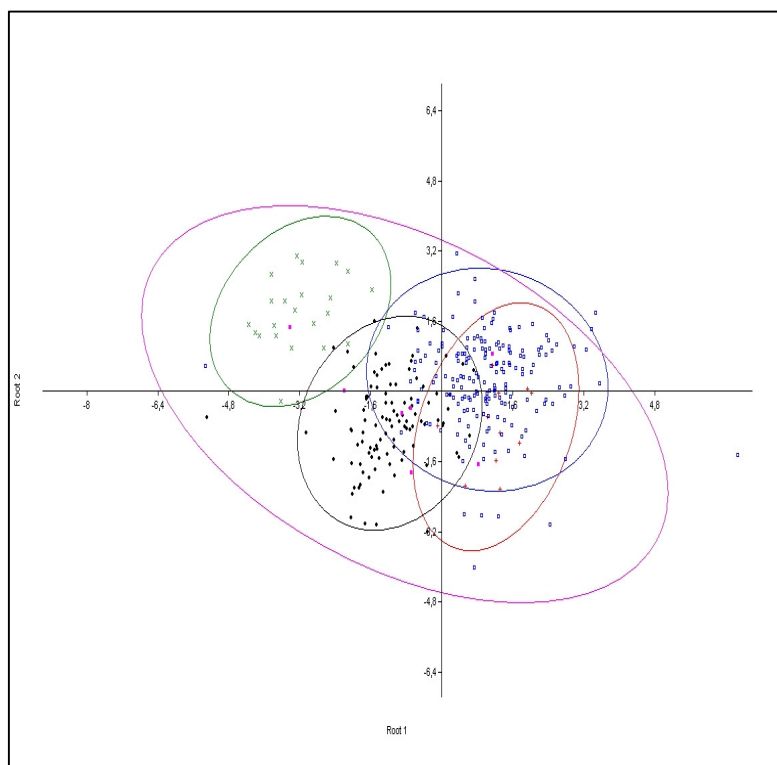
Figure 26: CVA scatterplot of the morphological variables and EFDs for *P. microps*: Bergen (●), Texel (+), Minho (□), Trondheim (■) and Skibotn/Sørbotn (x) fitted with 95% confidence ellipses

a2. Comparisons among seasons

In the comparison between seasons the MANOVA showed significant effects: Pillais' Trace=1,236; F= 10,75; p=2,558E-71. All pairwise comparisons revealed statistically significant. The Table with the Hotelling's T^2 tests is found on Appendix 33. The CVA scatterplot (Figure 27) shows some separation between the spring and the summer season due to higher scores on root 1 (associated with the variables 9, 17, 20) of the former and lower scores (mainly associated with variable 16) on the same axis of the later. Autumn and winter also show some degree of separation as a result of lower scores on canonical root 2 of the autumn season (associated with variables 13, 15 and 8) and higher scores on that same axis of the winter season (mainly associated with variables 16, otolith weight, variable 6, area, perimeter and Feret's diameter). The autumn season also shows relatively lower scores on root 2 that spring and summer seasons, while winter shows higher score in that axis when compared with summer and spring (Appendix 34, Figure 27).
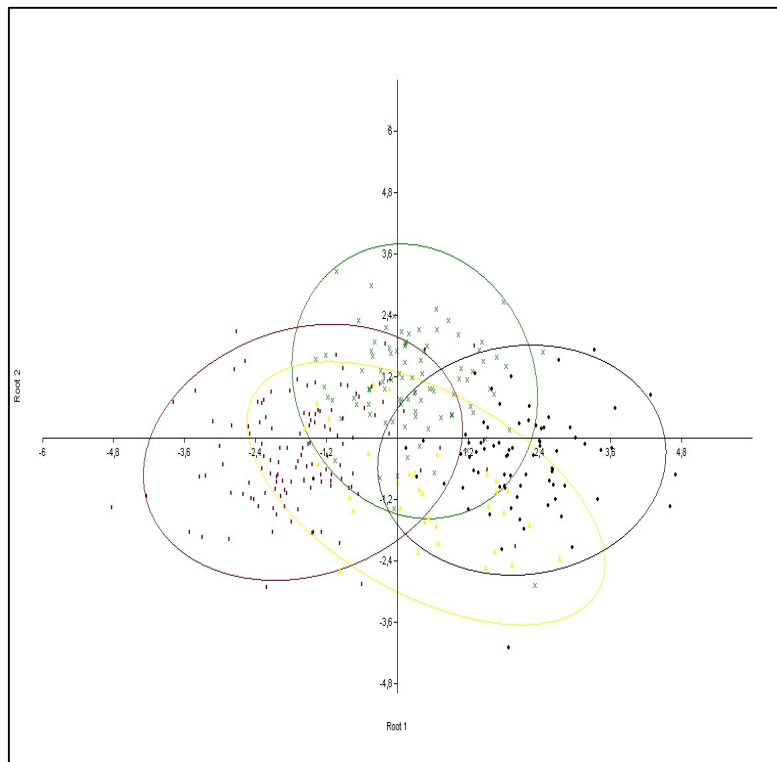
Figure 27: CVA scatterplot of the morphological variables and EFDs for *P. microps*: spring (x), summer (▲), autumn (ı) and winter (●) fitted with 95% confidence ellipses

b. *Pomatoschistus minutus*

b1. Comparisons among locations

The MANOVA comparing the different populations of the sand goby showed a significant result (Pillais' Trace=1,608; F= 6,992; p=1,205E-88) with the Hotelling's pairwise comparisons showing statistically significant differences between all pairs of populations (Appendix 35). The CVA scatterplot (Figure 28) allows for the partial separation of some pairs of populations: the population from Bergen can be separated from the population from Texel as a result of the lower scores on both the canonical root 1 (associated with circularity) and canonical root 2.
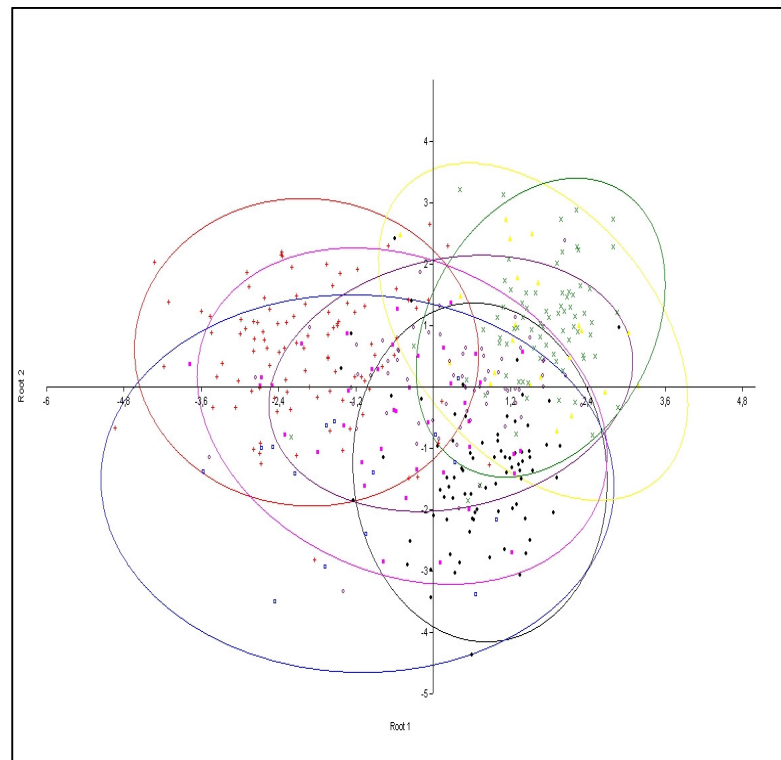
Figure 28: CVA scatterplot of the morphological variables and EFDs for *P. minutus*: Bergen (●), Texel (+), Minho (□), Valosen (▲), Trondheim (■) , Innhavet (○) and Skibotn/Sørbotn (x) fitted with 95% confidence ellipses

The specimens from Bergen are also marginally separated from the populations of Valosen and Skibotn/Sørbotn due to the lower scores on canonical root 1. Also, the populations from Valosen and Skibotn/Sørbotn can be separated from the specimens from

Texel due to the negative scores on the canonical root 2 of the latter (associated with otolith's weight, area and perimeter), while the first 2 have positive scores on this axis. The specimens from Trondheim, Innhavet and Minho are scattered throughout the plot (Appendix 36, Figure 28).

b2. Comparisons among seasons

Due to too few specimens caught during spring this season was not included in the analysis. In the comparison between seasons the MANOVA showed significant effects: Pillais' Trace=0,9039; F= 15,55; p=1,217E-73.
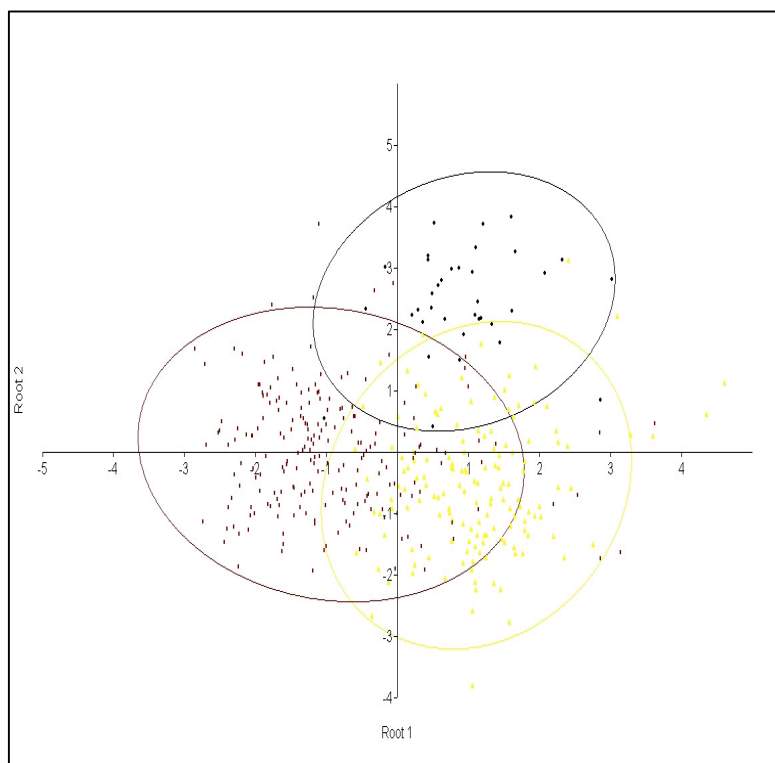
Figure 29: CVA scatterplot of the morphological variables and EFDs for *P. minutus*: summer (▲), autumn (|) and winter (●) fitted with 95% confidence ellipses

All pairwise comparisons revealed statistically significant. The Table with the Hotelling's T^2 tests is found on Appendix 37. The CVA scatterplot (Figure 29) shows some separation of the winter season from autumn and spring due to its higher score on root 2

(associated mainly with perimeter). Spring and autumn specimens also show some degree of separation due to negative scores on root 2 of the autumn specimens (with association with variables 17, 9 and circularity) and positive scores on the same axis of the summer specimens (mainly associated with otolith's weight and area) (Appendix 38, Figure 29).

IV. DISCUSSION

4.1 VARIATION IN OTOLITH SHAPE

With the development of image analysis systems and consequently of geometric morphometrics over the last 20 years the use of otolith shape in the identification of fish populations/stocks has become increasingly more popular (Tuset et al. 2003a, Adams et al. 2004, Turan 2004, Burke et al. 2008b). Nonetheless there are still some uncertainties about the effects of the environment and genetic background on its shape (Cardinale et al. 2004, Galley et al. 2006).

Interspecific variation

Several authors have referred the existence of interspecific variation in otolith shape (e.g. Lombarte & Castellón, 1991; Torres, 2000; Tuset et al., 2003a). It is argued that these differences are related to the hearing capacity of the fish since otoliths act as mechano-electrical receptors transducing sound and that the sound frequencies to which the otolith responds to depends on its shape (Popper & Coombs 1982, Gauldie 1988, Aguirre & Lombarte 1999, Morales-Nin 2000). In addition sex, age, year class and stock (Cardinale et al. 2004, Galley et al. 2006) are also reflected on their external morphology. Also, differences in metabolic rates have been linked to environmental factors that consequently might influence otolith shape (Popper & Coombs 1982, Wilson Jr 1985).

It is evident that there are some differences in the shape of the otoliths between these three species. Overall *Pomatoschistus pictus*' otoliths are smaller, lighter and are more circular while *Pomatoschistus minutus*' otoliths generally tend to be the less circular. *Pomatoschistus microps*' otoliths show intermediate characteristics between those two species. Despite these differences there is a high degree of overlap between them.

Gobies are known to emit sounds during the breeding season either as agonistic or courtship behavior. It has been suggested the existence of interspecific differences in those sounds functioning as a way of recognition between the species. Additionally, there is a correlation between these sounds and male body size (Lugli & Torricelli 1999, Lindström & Lugli 2000, Amorim & Neves 2007, 2008), as well as a direct relationship between the size of the otoliths and body size: the bigger the animal, the bigger the otolith (Arellano et al. 1995). This is in conformity with this study where *P. pictus*', being the smallest and less robust

species, proportionally has the smaller otoliths of the three species. However, the body size difference does not explain why *P. minutus*' otoliths are proportionally smaller than *P. microps*' otoliths when only the sample from Bergen is analyzed. Another possible explanation for the variation of not only the size but also the shape of the otoliths between these three species lies in possible differences in the food and spatial niches they occupy (Arellano et al. 1995, Aguirre & Lombarte 1999, Tuset et al. 2003a, Russo et al. 2008). The ratio between the *sulcus acusticus* and the otolith area (S:O) is known to be higher in fishes living in deeper waters than in fishes living and shallower waters with a benthic feeding mode, such as these three *Pomatoschistus* species (Gauldie 1988, Arellano et al. 1995). Although these gobies occupy very similar food and spatial niches there is still some degree of segregation due to competition but also because they occupy slightly different habitats. This resource partitioning has been linked to differences in body morphology (Russo et al. 2008) which might also lead to differences in the otoliths shape. On the one hand while the sand goby is commonly found in more saline and deeper waters the common goby generally inhabits more shallow waters, tolerating different levels of salinity values. On the other hand the painted goby inhabits gravel and sand and may even occur in tide pools. Food consumption has also been linked to the lobe formation in otoliths and it is indirectly related to its shape through somatic growth (Hüssy 2008). It is argued that with higher food consumption there is a higher deposition of protein which is likely to be responsible for the lobe formation. In part my results are in accordance with that hypothesis: the sand goby has shown the less circular otoliths of the three species but also the highest relative condition factor when all the populations were examined together. Although the sand goby has the less circular otoliths, in the comparison between these three species using only the population from Bergen the painted goby has the highest relative condition index indicating that there are other factors influencing the lobe formation in these otoliths.

What also becomes clear is that there is a "regional component" in the otolith shape. When these three species are compared using only the population from Bergen their discrimination is much lower with a higher degree of overlapping between them. In this case the common goby shows the largest and heavier otoliths but also the less circular. This might indicate a regional variation on otolith morphological features and general shape that might be the result of population stratification and therefore different gene pools or/and the influence of local environment. These results are accordingly to what has been argued in previous studies (Lombarte 1992, Arellano et al. 1995, Aguirre & Lombarte 1999, Tuset et al. 2003a)

that there is a basic evolutionary design shaping the otoliths belonging to a certain genus and the interspecific variation found within that genus is due to ecomorphological differences among the species.

Concluding, it is not possible to separate these three species based on otolith shape due to the influence that both genetics and environment have in it.

Intraspecific variation

Estuaries are highly geomorphologically dynamic habitats influenced by the ocean, rivers and land changes. They are a mosaic between several types of habitats (such as mudflats, salt marshes and lagoons) and have steep and sometimes unpredictable gradients in temperature, salinity, dissolved oxygen and turbidity (Attrill & Rundle 2002, Bilton et al. 2002, França et al. 2009). They also vary both geographically and seasonally and so it is expected that species inhabiting such an environmentally stressful zone (Attrill & Rundle 2002) are able to endure or avoid a wide range of values of those physical factors (Selleslagh & Amara 2008). As a consequence these estuarine populations are subjected to strong selection events (Pritchard 1967, Meire et al. 2005, Johannesson & André 2006, Nohrén et al. 2009) which coupled with specific local life history characteristics might enhance genetic and adaptive differences and lead to species stratification and consequently isolation between populations.

In species with such a broad distributional range such as these gobies temperature is one of the factors that varies the most between locations. Fish metabolism and growth rates are intimately connected to temperature variations therefore different populations are expected to show variations in otolith shape and weight (Campana & Neilson 1985, L'Abée-Lund 1988, Lombarte & Lleonart 1993, Galley et al. 2006). With lower growth rates otoliths tend to be heavier (Templeman & Squires 1956, Secor & Dean 1989, Tuset et al. 2003b). Although growth rates of the different populations have not yet been compared because populations from northern locations are subjected to lower temperatures it is expected that they will have lower growth rates (Barlow 1961). This would imply heavier otoliths for the population from Skibotn/Sørbotn and lighter otoliths for the population from Portugal. However, this is not observed in any of these two species. In the case of the common goby although the population from Minho does have the lighter otoliths the population from Bergen is the one showing the heavier otoliths, followed by the population from Texel and Skibotn/Sørbotn. In the case of the sand goby the northernmost population shows the

heaviest otoliths followed by the populations from Bodø and Bergen, with the population from Minho also having the lighter otoliths. These findings somewhat confirm that the northernmost populations do have lower growth rates which will give rise to heavier otoliths. Nevertheless there are still some exceptions to this latitudinal trend: the common gobies caught in Texel would be expected to have the second highest growth rate with consequently the second lightest otoliths, not only due to their latitude but also because this sample has been caught during the summer when the temperature and feeding conditions are ideal to promote a faster growth. This, however, does not happen and these animals show the second heaviest otoliths, which might indicate that some other factor is interfering. What also appears to be contradictory to what has been previously described is how otolith size (represented by its area) increases faster in the northernmost populations. This trend is valid for both the sand and the common goby. In fact the population from Minho shows the smallest increase in area of the otolith in relation to fish size in both species while Skibotn/Sørbotn shows the highest increase in size. Several studies have reported that otolith size is positively connected with temperature (see for example Lombarte & Lleonart 1993, Morales-Nin 2000) although Secor and Dean (Secor and Dean 1989) stated that slower growth rates will not only induce heavier but also larger otoliths. In the case of these two gobies none of the above hypothesis alone can explain the observed trends. This is also valid for circularity values: there is no clear relationship between the populations' relative condition factor and circularity and so, as stated above, Hüssy's (Hüssy 2008) hypothesis cannot be confirmed.

In conclusion both the sand and the common goby have a high degree of overlap between the different populations and so it is not possible to have a clear separation between them. Despite this overlap the common goby's population from Skibotn/Sørbotn is clearly separated from the population from Texel and has almost no overlapping with the population from Minho.

4.2 IS OTOLITH MORPHOLOGY POPULATION- OR SPECIES-SPECIFIC?

The three species chosen live sympatrically along the north-east Atlantic coast (Arellano et al. 1995). They occupy identical habitats, have similar diets and their life cycles are also alike (Bouchereau & Guelorget 1997, Amorim & Neves 2007). Furthermore their external and

otolith morphology is quite (Miller 1963) similar which makes them good candidates for this study.

Up to this moment no study has compared the otolith morphology of these species, although Arellano et al. (Arellano et al. 1995) has studied the variation of the *sulcus acusticus* area in relation to the sagitta area in *P. minutus*. He then compared these results with the results obtained for the also ecological and morphologically very similar *P. lozanoi*. Despite their similarity they detected differences in the allometric growth of the sagitta which were attributed to differences in the size of the skull and endolymohatic sac (that in turn is also related to body robustness). They hypothesized that the differences found are due to a niche segregation between the two species: the sand goby has a more benthic mode of feeding than the lozano's goby which explains the lower S:O ratio of the former. According to some authors the *sulcus acusticus* can be a good taxonomic tool (Torres et al. 2000).

In the case of these three species there is some degree of niche segregation which might contribute to a slightly different otolith shape, although the degree of separation between them is very low. It is important to compare otolith morphology with both the environmental variables and the genetic background of the individuals in order to clarify what are the mechanisms shaping otolith differences.

In conclusion the combination of all the analysis shows that there is a high degree of both inter- and intraspecific variability in the otolith shape of these species. This is a clear indication of interspecific affinity hampered by adaptations to local conditions of the different populations. As a consequence the separation of both species and populations cannot be made with certainty based solely on the otolith shape. As argued by Arellano et al. (Arellano et al. 1995) this might indicate that there is a basic evolutionary design in this genus with the variation found within the genus being related do differences in environmental factors. To elucidate which are the underlying mechanisms responsible for these differences there is the need for multiple-generation common-garden experiments where conditions can be controlled and adaptive plasticity can be distinguished from genetic background (Freitas 2011).

4.3 NATURE VS. NURTURE

Many fish species are composed by populations with various degrees of segregation between them (e.g. Burke et al., 2008; S. Campana & Casselman, 1993; Turan, 2004). This segregation is hypothesized to be determined by early life history events and it is related to speciation and extinction events, glaciation periods and the consequent variations in sea level but also by the species' tolerance to a number of factors such as temperature, salinity and dissolved oxygen (Gysels et al. 2004a, Gysels et al. 2004b, Soberon & Peterson 2005, Larmuseau et al. 2009b). Also shaping the population structure of these species are the oceanographic processes in the areas where spawning and larvae occur (Turrell 1992, Smedbol & Stephenson 2001, Gysels et al. 2004b, Galley et al. 2006). Along these lines I have followed the definition of *sub-population* from Smedbol and Stephenson (Smedbol & Stephenson 2001) and with *population* I mean "a semi-independent, self-reproducing group of individuals that undergo some measurable but limited exchange of individuals with other areas within the" species" range and thus may be genetically or phenotypically distinguishable from other" populations.

In species distributed along a wide latitudinal range their genetic structure is also influenced by marginality: populations from high latitudes are known to have reduced genetic variability (Gysels et al. 2004a, Johannesson & André 2006). The degree of variability is dependent upon the equilibrium between local selection pressures and connectivity with other populations (Johannesson & André 2006).

Several species have been reported to show differences among populations inhabiting different estuarine systems (Bilton et al. 2002). When analyzing the relationship between genetic and geographical distance *Pomatoschistus minutus*' populations from the northeast Atlantic coast do not exhibit a significant correlation between the two variables. Nevertheless when the variables are plotted there is a recognizable pattern of isolation-by-distance with populations from the southern North Sea grouping separately from populations of the Norwegian coast. When these groups were compared it was found that the interpopulational variation was higher than the variation found between populations (Gysels et al. 2004b). In the case of the common goby there is marked population stratification with a pattern of isolation-by-distance where the Mediterranean population is clearly separated from the Atlantic populations and the latter is divided in two groups, southern and northern Atlantic, with discontinuities around the British Isles (Gysels et al. 2004a, Berrebi et al. 2009). On the

one hand adults of both the sand and the common goby are known to be poor swimmers which limits the gene flow between the populations. On the other hand their eggs and larvae are planktonic which together with the existence of reproductive migrations to coastal areas (particularly in the sand goby) favors the maintenance of gene flow (Gysels et al. 2004a, Pampoulie et al. 2004, Gysels et al. 2004b). According to some studies species that occur in the upstream areas of the estuaries tend to have higher population stratification than species occurring closer to the estuary's mouth. This is thought to be connected with the potential for migration between estuaries (Bilton et al. 2002). Having in mind only the connectivity between estuaries it is expected that *P. microps'* populations show a higher degree of differentiation than its congener *P. minutus*. Nevertheless inferences based only in the genetic analysis have to be made carefully. In order to correctly interpret patterns in population stratification it is necessary to integrate genetic analysis with information on the ecology, history and demography of the species.

Condition factor

The relative condition index has been used in a variety of studies and therefore it is known to vary according to the environmental characteristics that are affecting the fish, the life history patterns of the species and the ecological interactions occurring in those habitats (Froese 2006). In this study *P. microps* and *P. minutus* were collected over a broad latitudinal range (spanning from 41,91ºN in Minho estuary, Portugal to 69,46ºN in Skibotn/Sørbotn, Norway) meaning that the different populations within a species were subjected to a vast range of environmental conditions (such as different temperatures and salinities, current speed, different pollution load and water quality, dissolved oxygen and photoperiod). The different sites of collection also reflect different life history patterns. Their high plasticity to environmental conditions leads to differences in the life cycle throughout their distributional range (Bouchereau & Guelorget 1997). These local differences in the life cycle might also be reflected in different migratory patterns and survival which also influence the condition of the fish (Bouchereau & Guelorget 1997, Froese 2006). In addition, several other factors might differ between the locations, with due consequences in the condition of the animals: different prey availability, parasite load and presence/absence of predators. It has also been documented that there is a social dominance of *P. minutus* over *P. microps* particularly when densities of both species are high and there is a spatial overlap and competition for resources(Edlund & Magnhagen 1981, Wiederholm 1987).

There was a big variation in the relative condition factor when comparing the values of all the populations merged and when only using the population from Bergen. This difference was particularly striking in the sand goby but could also be seen in the common goby. This variation indicates that in the case of these two species the environmental conditions (represented here by different sampling locations) play an important role in shaping the fitness of the fishes which leads to the need of studying the populations separately. Another factor that is important to have in consideration is that the relative condition factor of the animals increases with total length. In both cases described above the highest average K_{rel} also matches the highest average total length.

In the case of *Pomatoschistus microps* the highest relative condition factor was found in the northernmost location (at 69,46ºN in Skibotn/Sørbotn, Norway) followed by the southernmost population (at 41,91ºN in Minho estuary, Portugal) while the lowest relative condition factor was found in Bergen. When these samples were divided in seasons (and as expected) the summer season showed the highest relative condition factor followed by autumn, spring and finally winter. The low relative condition factor of the individuals from Bergen might be explained by the fact that these samples were collected during mid-autumn and winter, while the sample from Skibotn/Sørbotn was collected during summer and early autumn and the sample from Minho during spring and summer. During winter the food availability for these species is lower and so is their food intake (Healey 1972, Fouda & Miller 1981). However their diet also varies between locations which means that they should rarely encounter periods of complete starvation and therefore their lower intake of food might have a stronger link with lower temperatures that reduce their metabolism and consequently their food intake. This would lead to a usage of the fat reserves which can be translated into a lower condition (Lindsey 1966, Healey 1972). Another possibility connected to time of the year is that in northern locations the photoperiod is much shorter during winter. As sit-and-wait visual predators the gobies would have a shorter time frame to capture prey. This situation could also lead to a higher frequency of movements in order to search for prey which would make them more vulnerable to predators. Even so, seasonality alone does not explain the differences in the relative condition factor between the populations since also the population from Texel was caught during the summer and the population from Trondheim during early autumn. To explain this variation several scenarios might be hypothesized: bigger animals are known to have higher K_{rel} (Fulton 1904) and the specimens from Skibotn/Sørbotn have the highest average total length while the animals from Bergen show the lowest average total

length. Also the common goby is better adapted to warmer temperatures (Fonds & Van Buurt 1974) which might explain why the population from Minho shows the second highest K_{rel}. Another possible explanation lies in the fact that fish condition might reflect the condition of the habitat where it resides indicating its health status (Holt & Miller 2011). Another plausible explanation lies in the existence of interspecific competition between these two species. *P. microps* is known to be socially dominated by *P. minutus* (Edlund & Magnhagen 1981). In the sample from Texel the density of the common goby was much smaller than that of the sand goby which might limit their access to food. This interspecific competition would also explain the much higher relative condition factor values of the sand goby in all the populations.

When all the populations of *Pomatoschistus minutus* were compare the results were different from those of *P. microps*: by far the highest condition factor was found in the population from Texel followed by the population from Bodø, although with a very big difference between the two values. As in the case of the common goby the population from Bergen had the lowest relative condition value. When the specimens were divided in seasons the specimens collected during the summer months revealed a much higher relative condition factor that was followed by autumn and winter. There is a noteworthy difference in the condition factor of the sample from Texel. This high value might be explained by the fact that the average size of these specimens was much higher than those of the remaining populations.

The seasonal differences found in the condition might be explained by the same hypothesis shown above.

4.4 FUTURE RESEARCH

This study does not answer which are the driving forces shaping the otoliths in these species. Further studies are needed in order to disentangle to which extent there is a genetic control behind the otoliths' shape and how environmental factors are also playing a part in it. In order to accomplish this common-garden experiments should be planned where multiple generations can be reared in a controlled environment under the influence of various levels of temperature, photoperiod and food consumption. In this way the the underlying mechanisms responsible for these differences can be elucidated, distinguishing adaptive plasticity effects from the influence of the genetic background of the specimens. Also the effects of food consumption, metabolic and growth rates can be understood.

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LENGTH-WEIGHT RELATIONSHIPS, RELATIVE CONDITION FACTOR AND CUMULATIVE CONDITION DISTRIBUTIONS (CCDS)

Interspecific comparisons

a. ALL POPULATIONS MERGED

APPENDIX 1

Kruskal-Wallis test results for the interspecific CCDs. Significant differences are shown in bold.

hC		9,176
Hc		9,352
p(same)		**0,01017**
Post-hoc pairwise tests		

0	P. microps	P. minutus	P. pictus
P. microps	0	**0,003204**	0,3578
P. minutus	0,009612	0	**0,04117**
P. pictus	1	0,1235	0

b. BERGEN

APPENDIX 2

Kruskal-Wallis test results for the interspecific CCDs in Bergen. Significant differences are shown in bold.

H	2,935
Hc	2,992
p(same)	0,2305

Intraspecific comparisons

a. POMATOSCHISTUS MICROPS

a1. COMPARISONS BETWEEN POPULATIONS

APPENDIX 3

Kruskal-Wallis test results for the different populations of *P. microps*. Significant differences are shown in bold.

H	2,12E+02
Hc	2,12E+02
p(same)	**1,08E-44**

	Bergen	Texel	Minho	Trondheim	Skibotn/Sørbotn
Bergen	0	**1,44E-05**	**1,63E-19**	**1,52E-07**	**2,14E-30**
Texel	0,000144	0	**5,01E-12**	**3,12E-05**	**5,04E-22**
Minho	1,63E-18	5,01E-11	0	0,008312	**3,88E-18**
Trondheim	1,52E-06	0,000312	0,08312	0	**5,36E-13**
Skibotn/Sørbotn	2,14E-29	5,04E-21	3,88E-17	5,36E-12	0

a2. COMPARISONS BETWEEN SEASONS

APPENDIX 4

Kruskal-Wallis test results for the different seasons of *P. microps*. Significant differences are shown in bold.

H	95,22
Hc	95,41
p(same)	**1,66E-20**

spring	summer	autumn	winter
0	**6,34E-08**	**0,003605**	**1,51E-06**
3,80E-07	0	**6,11E-11**	**1,09E-15**
0,02163	3,66E-10	0	**8,28E-09**
9,06E-06	6,51E-15	4,97E-08	0

b. _Pomatoschistus minutus_

b1. COMPARISONS BETWEEN POPULATIONS

APPENDIX 5

Kruskal-Wallis test results for the different populations of _P. minutus_. Significant differences
are shown in bold.

H	3,39E+02
Hc	3,40E+02
p(same)	**3,81E-70**

0	Bergen	Texel	Minho	Valosen	Trondheim	Innhavet	Skibotn/Sørbotn
Bergen	0	**2,14E-28**	**7,57E-08**	**0,001183**	**2,68E-10**	**2,25E-12**	**2,41E-11**
Texel	4,50E-27	0	**3,87E-22**	**1,58E-24**	**1,82E-24**	**4,34E-15**	**4,36E-34**
Minho	1,59E-06	8,12E-21	0	**1,29E-05**	**0,009021**	**0,000337**	**3,45E-19**
Valosen	0,02484	3,32E-23	0,000271	0	**1,26E-09**	**1,88E-11**	**1,59E-20**
Trondheim	5,63E-09	3,83E-23	0,1894	2,64E-08	0	**0,0342**	**4,25E-20**
Innhavet	4,72E-11	9,12E-14	0,007074	3,94E-10	0,7183	0	**2,50E-19**
Skibotn/Sørbotn	5,06E-10	9,16E-33	7,24E-18	3,34E-19	8,92E-19	5,24E-18	0

b2. COMPARISONS BETWEEN SEASONS

APPENDIX 6

Kruskal-Wallis test results for the different seasons of *P. minutus*. Significant differences are shown in bold.

H	515,3
Hc	516,1
p(same)	**1,24E-112**

0	summer	autumn	winter
summer	0	**5,96E-65**	**1,38E-82**
autumn	1,79E-64	0	**3,30E-33**
winter	4,13E-82	9,89E-33	0

OTOLITH MORPHOLOGICAL PATTERNS AND ITS RELATIONSHIP WITH
OTOLITH SIZE

In this section the abreviations used are the following: TL – total length of the fish; A –
otolith's area; Perim – otolith's perimeter; Circ – otolith's circularity; Feret – Feret's diameter

Interspecific comparisons

a. ALL POPULATIONS MERGED

APPENDIX 7

Number (n), average (Avg), standard deviation (SD), maximum (max) and minimum (min)
values of the morphological variables for *Pomatoschistus microps*

	n	Avg	SD	max	min
TL (mm)	536	33,62	5,38	50,00	21,00
A (mm^2)	418	0,8305	0,21438	1,8132	0,2639
Perim (mm)	412	3,28107	0,45689	4,867	1,8435
Circ	418	0,95159	0,01956	0,985	0,854
Feret (mm)	411	1,09525	0,15518	1,504	0,619
Wot (g)	461	0,51003	0,19021	1,331	0,102

Number (n), average (Avg), standard deviation (SD), maximum (max) and minimum (min)
values of the morphological variables for *Pomatoschistus minutus*

	n	Avg	SD	max	min
TL (mm)	592	40,96	10,15	66,00	20,50
A (mm^2)	478	1,04325	0,47583	2,5975	0,2129
Perim (mm)	480	3,66981	0,92715	5,9712	1,6649
Circ	468	0,93512	0,03865	0,988	0,822
Feret (mm)	477	1,21184	0,30506	1,955	0,555
Wot (g)	466	0,7356	0,43073	2,189	0,091

Number (n), average (Avg), standard deviation (SD), maximum (max) and minimum (min) values of the morphological variables for *Pomatoschistus pictus*

	n	Avg	SD	max	min
TL (mm)	62	33,92	5,04	44,00	26,00
A (mm^2)	68	0,71281	0,17656	1,1283	0,4838
Perim (mm)	68	3,00992	0,48644	4,213	0,8581
Circ	68	0,96072	0,01892	0,984	0,88
Feret (mm)	61	0,98597	0,12427	1,3	0,824
Wot (g)	67	0,44985	0,17524	0,934	0,246

APPENDIX 8

Linear regressions for *P. microps*. Significant differences are shown in bold.

Linear regressions	n	Slope	Intercept	r^2	p-value
log TL – log A	402	1,7701	-2,7885	0,61055	6,26E-84
log A – log Perim	412	0,51593	0,5621	0,99531	0
TL – Circ	339	-0,0038	1,0805	0,14314	5,60E-13
TL – Feret	396	0,02973	0,09982	0,60175	8,81E-81
TL - Wot	446	0,03582	-0,7008	0,64885	5,91E-103

Linear regressions for *P. minutus*. Significant differences are shown in bold.

Linear regressions	n	Slope	Intercept	r^2	p-value
log TL – log A	457	1,9287	-3,117	0,78724	**5,26E-155**
log A – log Perim	475	0,53549	0,566	0,99576	**0**
TL – Circ	421	-0,004	1,0964	0,46888	**1,52E-59**
TL – Feret	459	0,03011	-0,0273	0,80069	**3,65E-162**
TL - Wot	453	0,04255	-1,0406	0,82992	**1,34E-175**

Linear regressions for *P. pictus*. Significant differences are shown in bold.

Linear regressions	n	Slope	Intercept	r²	p-value
log TL – log A	57	1,5403	-2,5159	0,68916	**1,42E-15**
log A – log Perim	65	0,52614	0,56257	0,99678	**0**
TL – Circ	40	-0,0025	1,0514	0,45426	**1,87E-06**
TL – Feret	52	0,0255	0,12971	0,69375	**1,90E-14**
TL - Wot	58	0,0342	-0,7297	0,66285	**7,77E-15**

APPENDIX 9

ANCOVA table of the different regressions compared between *P. microps*, *P. minutus* and *P. pictus*. Significant differences are shown in bold.

log TL – log A

	SS	df	MS	F	p(same)
Adj. means	0,79912	2	0,39956	53,65	**9,34E-23**
Adj. error	6,7917	912	0,007447		
Adj. total	7,5908	914			
Homogeneity of slopes					
F:	11,99				
p(equal):	**7,25E-06**				

log A – log Perim

	SS	df	MS	F	p(same)
Adj. means	0,001355	2,00E+00	6,77E-04	18,14	**1,85E-08**
Adj. error	3,54E-02	948	3,73E-05		
Adj. total	3,67E-02	950			
Homogeneity of slopes					
F:	26,52				
p(equal):	**6,20E-12**				

TL – Circ

	SS	df	MS	F	p(same)
Adj. means	0,007866	2	0,003933	6,942	**0,001026**
Adj. error	0,451	7,96E+02	5,67E-04		
Adj. total	4,59E-01	798			
Homogeneity of slopes					
F:	11,07				
p(equal):	**1,81E-05**				

TL – Feret

	SS	df	MS	F	p(same)
Adj. means	1,4915	2,00E+00	7,46E-01	53,52	**1,08E-22**
Adj. error	1,26E+01	903	1,39E-02		
Adj. total	1,41E+01	9,05E+02			
Homogeneity of slopes					
F:	5,657				
p(equal):	**0,00362**				

TL – Wot

	SS	df	MS	F	p(same)
Adj. means	0,90935	2	0,45468	20,46	**1,99E-09**
Adj. error	21,174	9,53E+02	2,22E-02		
Adj. total	22,083	955			
Homogeneity of slopes					
F:	25,27				
p(equal):	**2,02E-11**				

Linear relationships between the different variables for *P. microps*, *P. minutus* and *P. pictus*.
P. microps (●), *P. minutus* (+), *P. pictus* (□)

logTL – log A
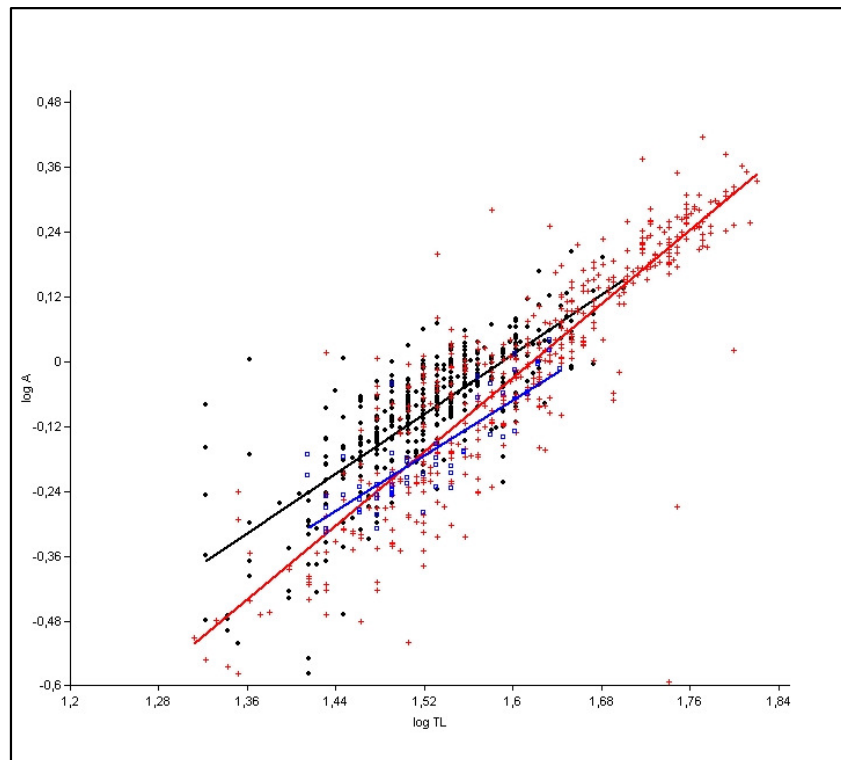
Log A – log Perim

TL – Circ
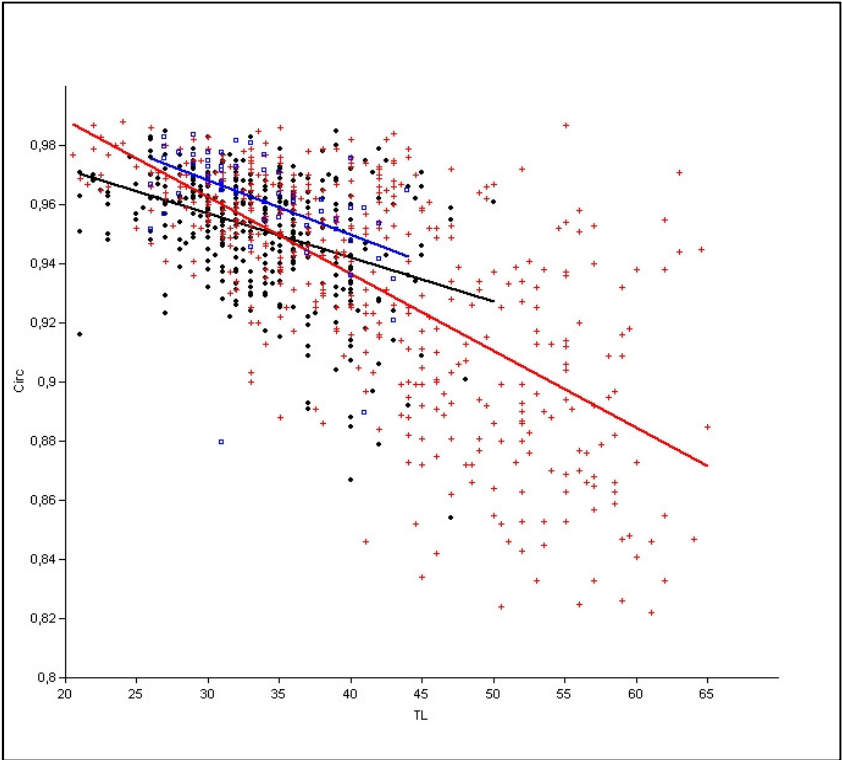
TL – Feret
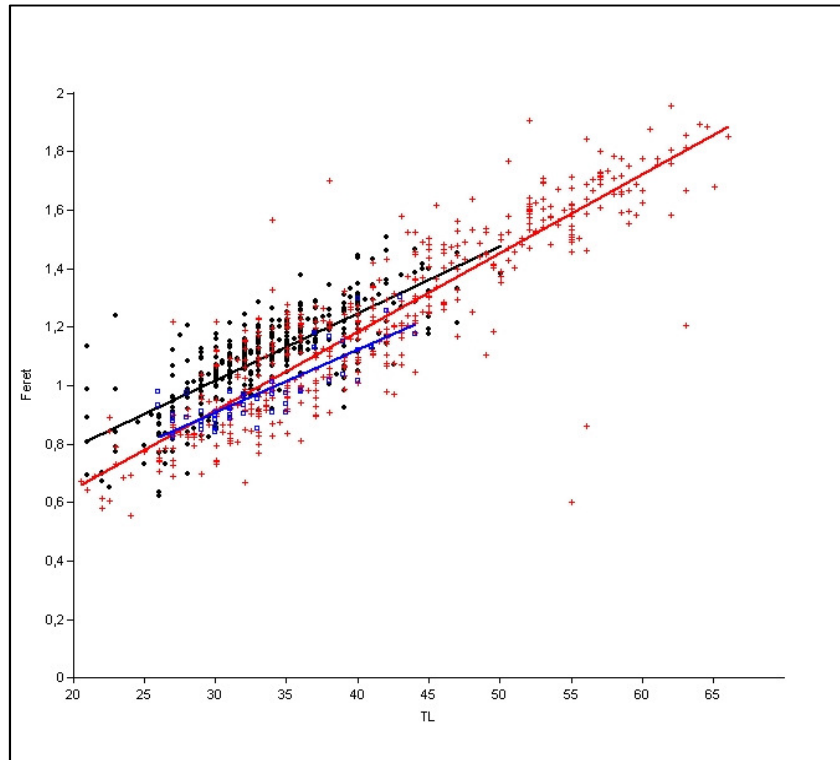
TL - Wot
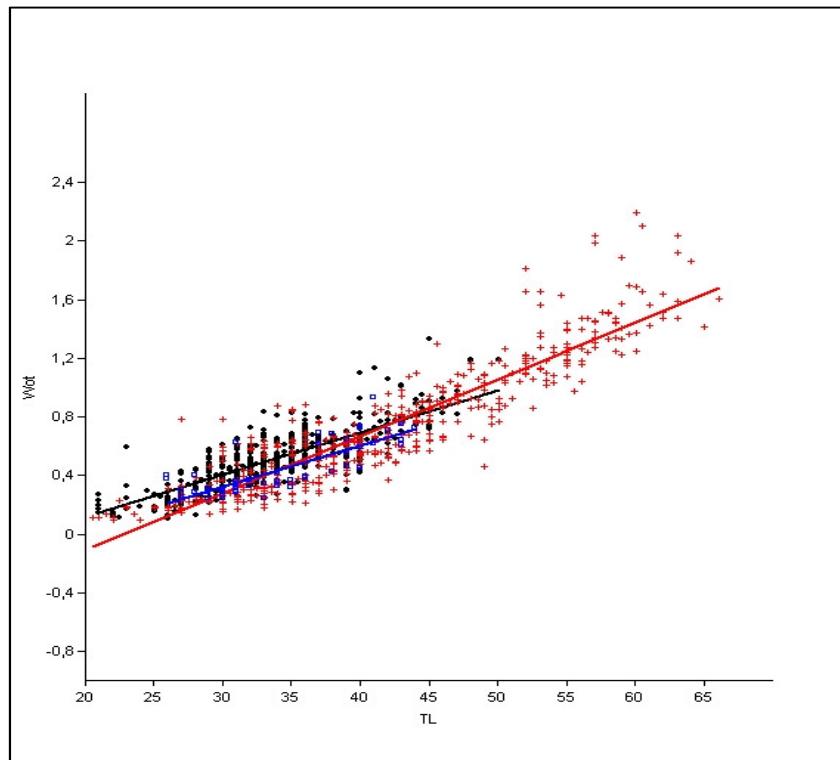
APPENDIX 11

Number (n), average (Avg), standard deviation (SD), maximum (max) and minimum (min)
values of the morphological variables for *Pomatoschistus microps* caught in Bergen. TL –
total length; A – area; Perim – perimeter; Circ – circularity; Feret – Feret's diameter; Wot –
otolith weight;

	n	Avg	SD	max	min
TL (mm)	130	31,29	3,94	43,00	21,00
A (mm^2)	115	0,79	0,22	1,32	0,30
Perim (mm)	109	3,16	0,49	4,16	1,98
Circ	116	0,96	0,01	0,99	0,94
Feret (mm)	111	1,05	0,16	1,38	0,65
Wot (g)	126	0,50	0,19	1,01	0,11

Number (n), average (Avg), standard deviation (SD), maximum (max) and minimum (min)
values of the morphological variables for *Pomatoschistus minutus* caught in Bergen. TL –
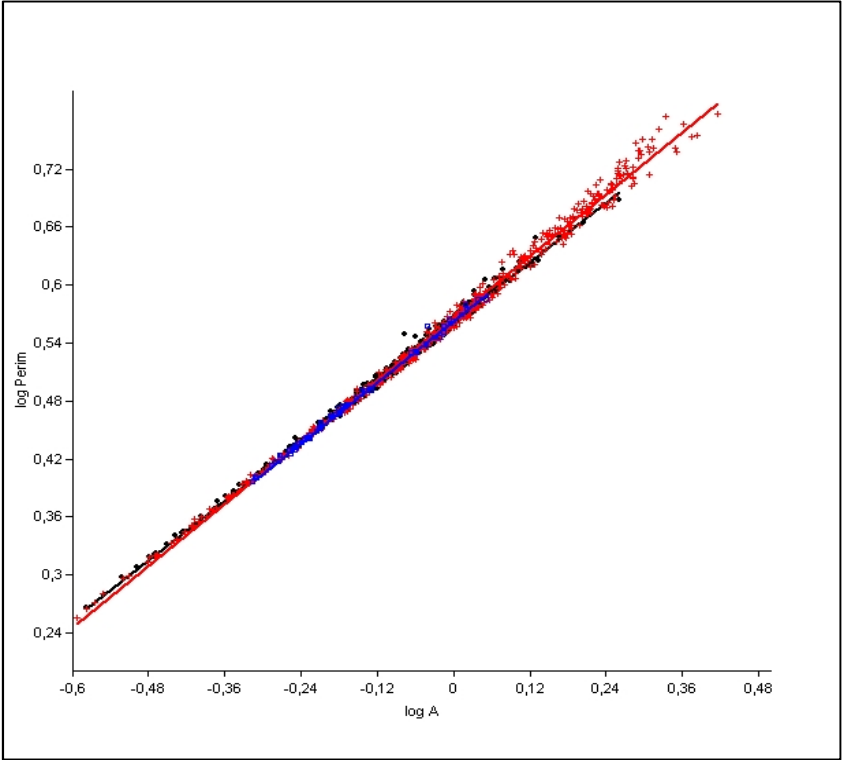total length; A – area; Perim – perimeter; Circ – circularity; Feret – Feret's diameter; Wot –
otolith weight;

	n	Avg	SD	max	min
TL (mm)	102	33,43	7,78	62,00	20,50
A (mm^2)	101	0,86	0,43	2,60	0,28
Perim (mm)	103	3,28	0,79	5,97	1,91
Circ	101	0,96	0,02	0,98	0,88
Feret (mm)	100	1,08	0,25	1,96	0,64
Wot (g)	92	0,51	0,31	2,12	0,11

Number (n), average (Avg), standard deviation (SD), maximum (max) and minimum (min) values of the morphological variables for *Pomatoschistus pictus* caught in Bergen. TL – total length; A – area; Perim – perimeter; Circ – circularity; Feret – Feret's diameter; Wot – otolith weight;

	n	Avg	SD	max	min
TL (mm)	62	33,92	5,04	44,00	26,00
A (mm^2)	68	0,71	0,18	1,13	0,48
Perim (mm)	68	3,01	0,49	4,21	0,86
Circ	68	0,96	0,02	0,98	0,88
Feret (mm)	61	0,99	0,12	1,30	0,82
Wot (g)	67	0,45	0,18	0,93	0,25

APPENDIX 12

Linear regressions for *P.microps* caught in Bergen. Significant differences are shown in bold.

Linear regressions	n	Slope	Intercept	r^2	p-value
log TL – log A	105	2,5583	-3,9424	0,7063	**3,68E-29**
log A – log Perim	109	0,50427	0,55833	0,99913	**1,54E-165**
TL – Circ	105	-0,0025	1,0401	0,10488	**0,00075161**
TL – Feret	105	0,04119	-0,2415	0,67798	**4,30E-27**
TL - Wot	103	0,04823	-1,0126	0,70543	**1,47E-28**

Linear regressions for *P.minutus* caught in Bergen. Significant differences are shown in bold.

Linear regressions	n	Slope	Intercept	r^2	p-value
log TL – log A	89	2,1016	-3,3056	0,81698	**7,80E-34**
log A – log Perim	101	0,5128	0,56107	0,99861	**2,62E-143**
TL – Circ	89	-0,00242	1,0357	0,5321	**5,19E-16**
TL – Feret	88	0,033608	-0,05235	0,82616	**2,00E-34**
TL - Wot	83	0,040163	-0,819	0,63064	**3,35E-19**

Linear regressions for *P.pictus* caught in Bergen. Significant differences are shown in bold.

Linear regressions	n	Slope	Intercept	r^2	p-value
log TL – log A	56	1,5373	-2,5111	0,7063	**3,32E-15**
log A – log Perim	68	0,52614	0,56257	0,99678	**3,30E-80**
TL – Circ	57	-0,00393	1,0938	0,22384	**0,00020191**
TL – Feret	53	0,025503	0,12971	0,69375	**1,90E-14**
TL - Wot	57	0,034725	-0,74597	0,65308	**5,11E-14**

APPENDIX 13

ANCOVA table of the different regressions compared between *P. microps*, *P. minutus* and *P. pictus* from Bergen. Significant differences are shown in bold.

log TL – log A

	SS	df	MS	F	p(same)
Adj. means	0,4124	2	0,2062	32,16	**3,58E-13**
Adj. error	1,622	253	0,00641		
Adj. total	2,0344	255			
Homogeneity of slopes					
F:	8,729				
p(equal):	**0,00022**				

log A – log Perim

	SS	df	MS	F	p(same)
Adj. means	0,00251	2	0,00126	1,357	0,2591
Adj. error	0,25358	274	0,00093		
Adj. total	0,2561	276			
Homogeneity of slopes					
F:	3,402				
p(equal):	**0,03474**				

TL – Circ

	SS	df	MS	F	p(same)
Adj. means	0,00209	2	0,00104	6,041	**0,00274**
Adj. error	0,04388	254	0,00017		
Adj. total	0,04597	256			
Homogeneity of slopes					
F:	3,549				
p(equal):	**0,03021**				

TL – Feret

	SS	df	MS	F	p(same)
Adj. means	0,58259	2	0,2913	30,8	**1,20E-12**
Adj. error	2,2982	243	0,00946		
Adj. total	2,8808	245			
Homogeneity of slopes					
F:	6,023				
p(equal):	**0,0028**				

TL – Wot

	SS	df	MS	F	p(same)
Adj. means	0,93277	2	0,46638	16,86	**1,30E-07**
Adj. error	7,1922	260	0,02766		
Adj. total	8,125	262			
Homogeneity of slopes					
F:	3,186				
p(equal):	**0,04297**				

APPENDIX 14

Linear relationships between the different variables for *P. microps*, *P. minutus* and *P. pictus* from Bergen

Log TL – log A
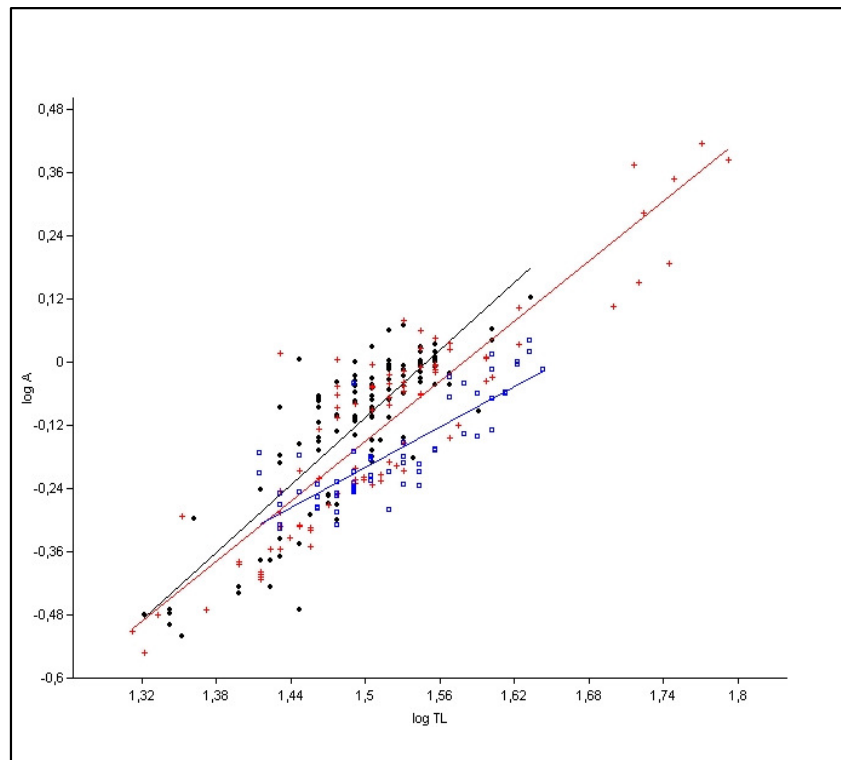
Log A – log Perim

TL – Circ

TL - Feret

TL - Wot

Intraspecific comparisons

a. *Pomatoschistus microps*
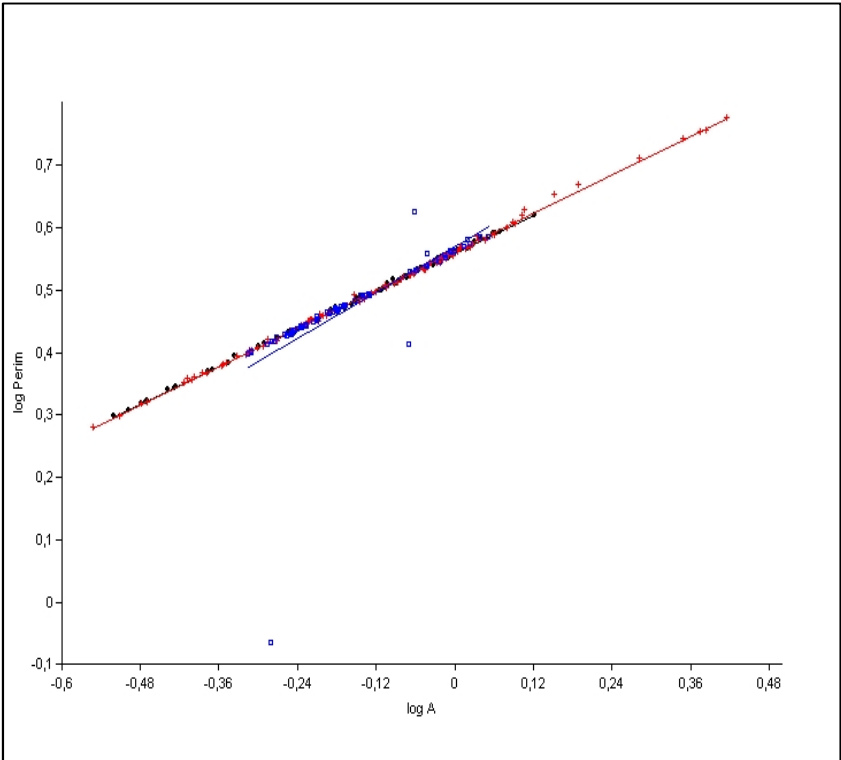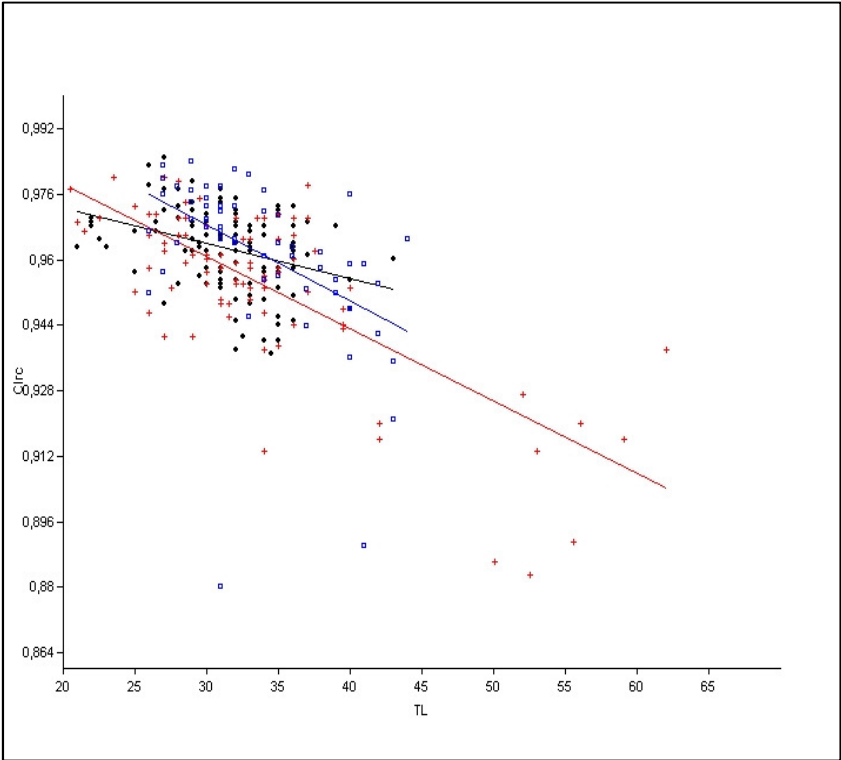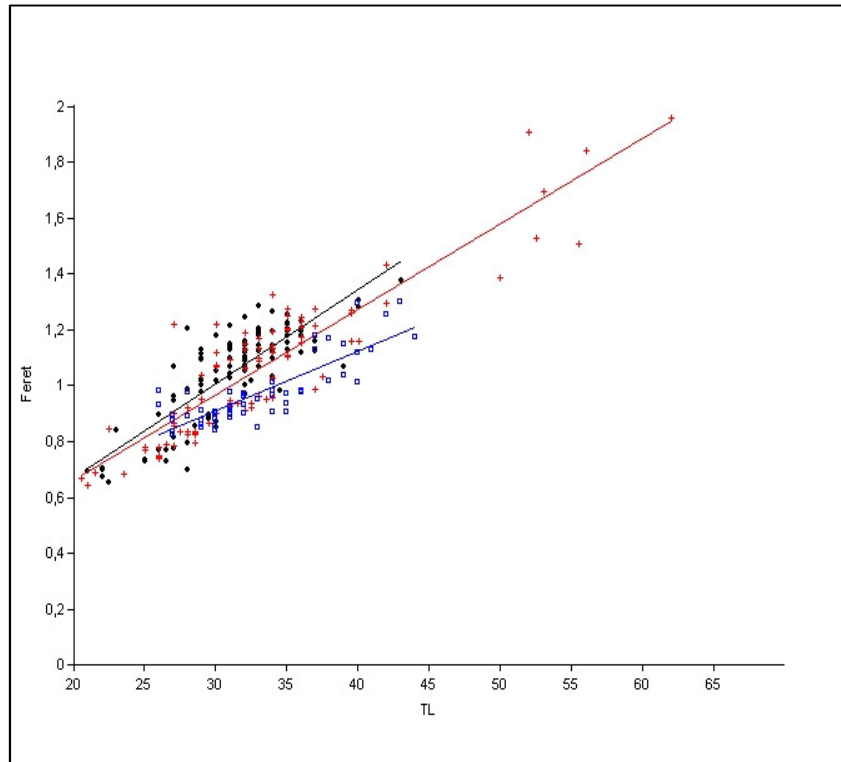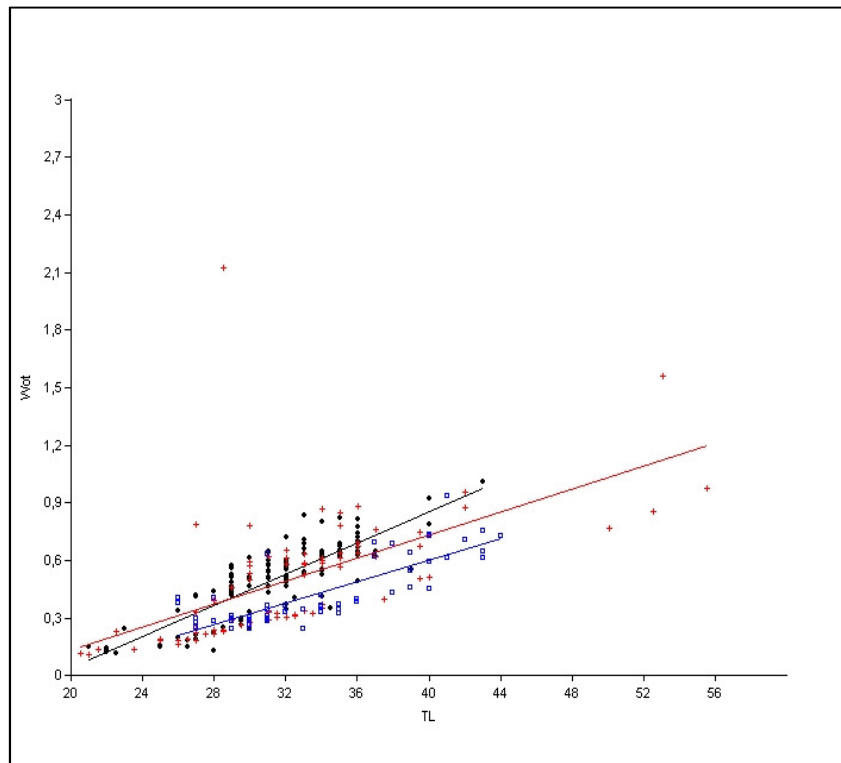
a1. COMPARISONS BETWEEN POPULATIONS

APPENDIX 15

Linear regressions for the different populations of *P. microps*. Significant differences are shown in bold.

log TL – log A

Populations	n	Slope	Intercept	r^2	p-value
Bergen	111	2,5523	-3,931	0,70151	**2,20E-30**
Texel	13	1,8151	-2,8457	0,6914	**0,00043**
Minho	192	1,4418	-2,287	0,58632	**2,88E-38**
Trondheim	8	2,0659	-3,2938	0,87323	**0,00067**
Skibotn/Sørbotn	22	2,1789	-3,5599	0,84749	**1,29E-09**

log A – log Perim

Populations	n	Slope	Intercept	r^2	p-value
Bergen	109	0,50427	0,55833	0,99913	**1,54E-165**
Texel	13	0,53789	0,56637	0,99448	**8,96E-14**
Minho	196	0,52233	0,56371	0,99278	**103E-209**
Trondheim	9	0,53801	0,56906	0,99607	**1,11E-09**
Skibotn/Sørbotn	22	0,50699	0,55639	0,99948	**2,50E-34**

TL – Circ

Populations	n	Slope	Intercept	r^2	p-value
Bergen	112	-0,0025	1,0414	0,11851	**0,0002**
Texel	13	-0,0058	1,1291	0,21818	0,10756
Minho	191	-0,004	1,0839	0,22705	**3,22E-12**
Trondheim	8	-0,0063	1,1475	0,33318	0,13409
Skibotn/Sørbotn	22	-154	1,0331	0,37743	**0,00235**

TL – Feret

Populations	n	Slope	Intercept	r^2	p-value
Bergen	107	0,04105	-0,2348	0,66853	**6,30E-27**
Texel	13	0,03267	0,0218	0,65603	**0,00079**
Minho	191	0,0275	0,18424	0,57535	**5,37E-37**
Trondheim	8	0,03427	-0,1209	0,85413	**0,00103**
Skibotn/Sørbotn	22	0,02883	-0,055	0,84206	**1,84E-09**

TL – Wot

Populations	n	Slope	Intercept	r^2	p-value
Bergen	121	0,04863	-1,0232	0,69747	**1,11E-32**
Texel	11	0,03921	-0,8328	0,52262	**0,01195**
Minho	200	0,03087	-0,571	0,59904	**3,71E-41**
Trondheim	7	0,03519	-0,7434	0,87342	**0,00203**
Skibotn/Sørbotn	26	0,03661	-0,8027	0,86101	**8,97E-12**

APPENDIX 16

ANCOVA table of the different regressions compared between *P. microps*' populations.
Significant differences are shown in bold.

log TL – log A

	SS	df	MS	F	p(same)
Adj. means	0,24059	4	0,060148	11,41	**1,06E-08**
Adj. error	1,7921	340	0,005271		
Adj. total	2,0327	344			
Homogeneity of slopes					
F:	16,73				
p(equal):	**1,63E-12**				

log A – log Perim

	SS	df	MS	F	p(same)
Adj. means	0,001446	4	0,000362	24,46	**7,91E-18**
Adj. error	0,00507	343	1,48E-05		
Adj. total	0,006516	347			
Homogeneity of slopes					
F:	6,463				
p(equal):	**5,06E-05**				

TL – Circ

	SS	df	MS	F	p(same)
Adj. means	0,031293	4	0,007823	35,76	**6,08E-25**
Adj. error	0,07438	340	0,000219		
Adj. total	0,10567	344			
Homogeneity of slopes					
F:	2,352				
p(equal):	0,05385				

TL – Feret

	SS	df	MS	F	p(same)
Adj. means	0,57474	4	0,14369	17,98	**2,19E-13**
Adj. error	2,6776	335	0,007993		
Adj. total	3,2523	339			
Homogeneity of slopes					
F:	6,74				
p(equal):	**3,17E-05**				

TL – Wot

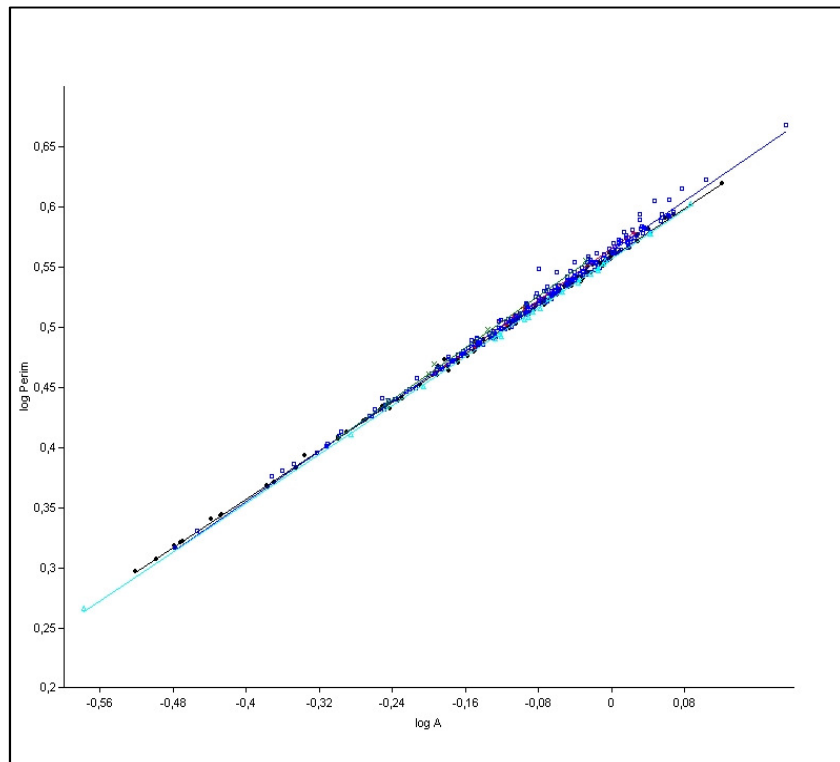|	SS	df	MS	F	p(same)
Adj. means	0,78026	4	0,19507	19,24	**2,37E-14**
Adj. error	3,6399	359	0,010139		
Adj. total	4,4202	363			
Homogeneity of slopes					
F:	10,37				
p(equal):	**5,88E-08**				

APPENDIX 17

Linear relationships between the different variables for *P. microps*' populations. Bergen (●), Texel (+), Minho (□), Trondheim (x) and Skibotn/Sørbotn (Δ)
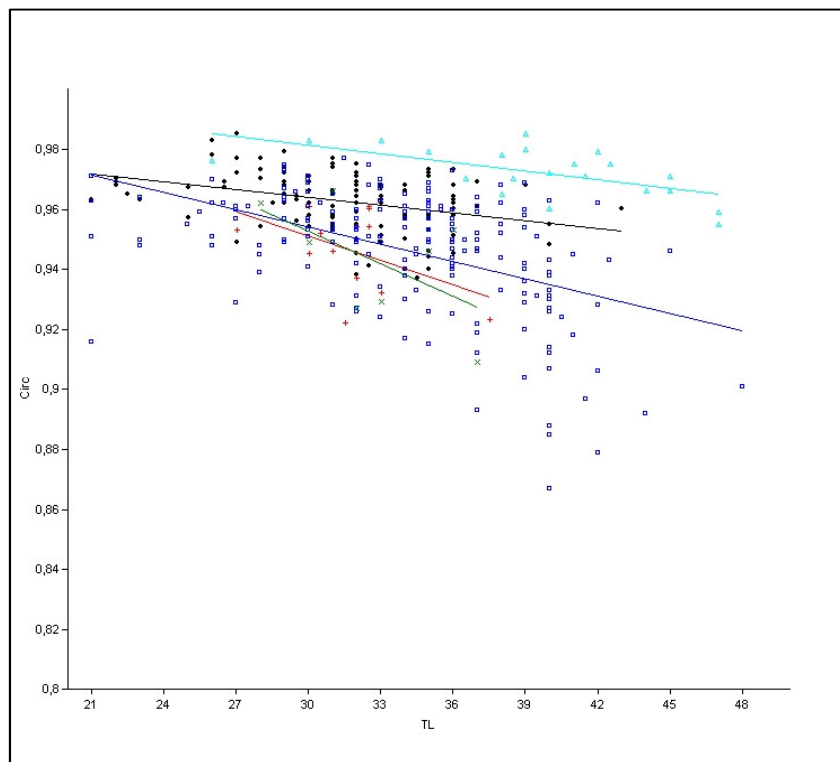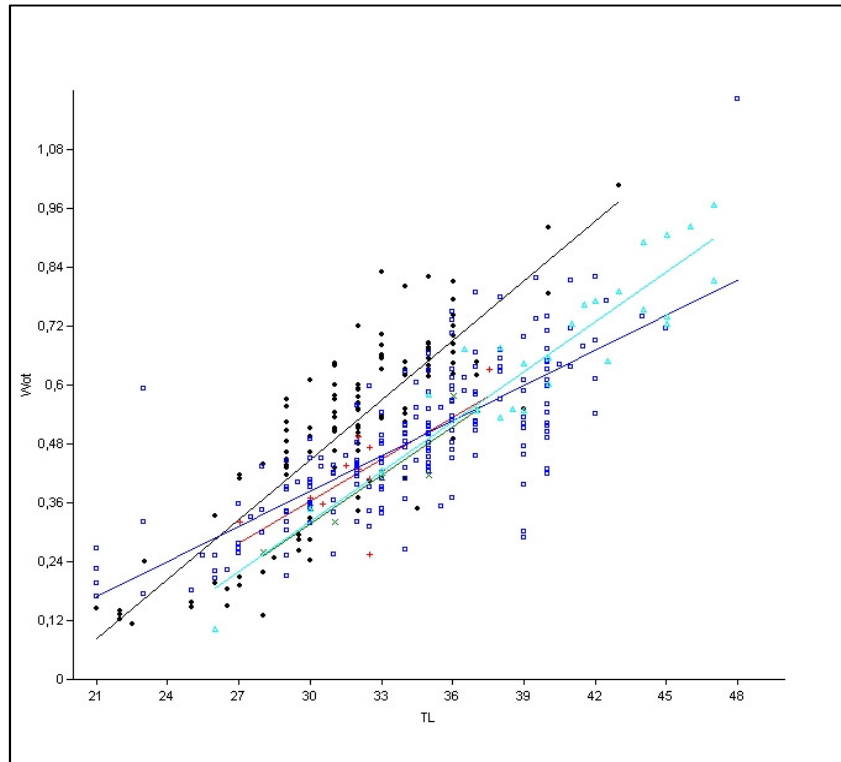
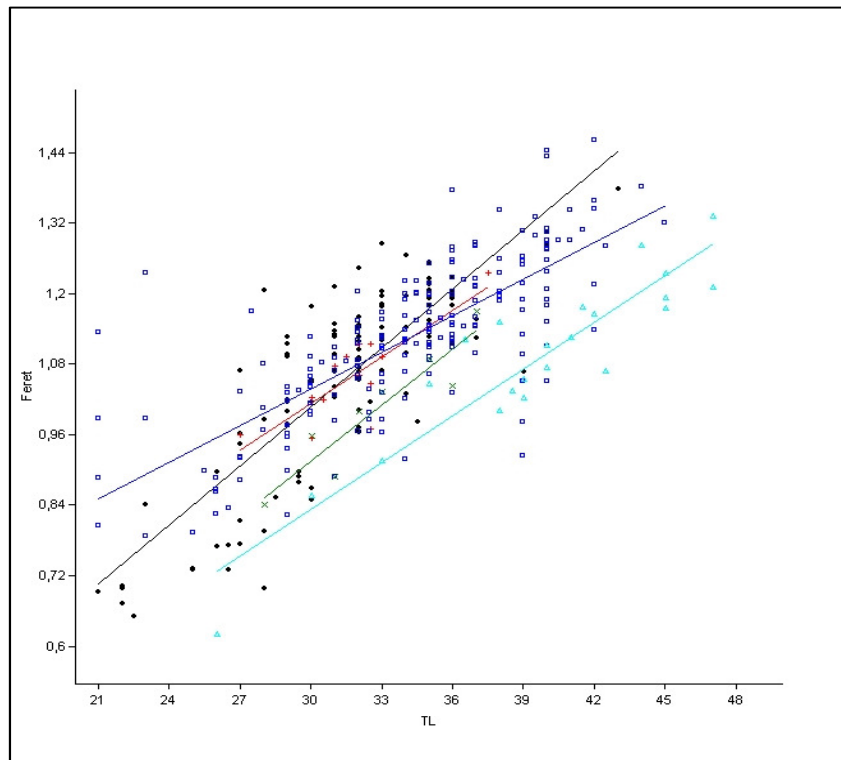Log TL – Log A
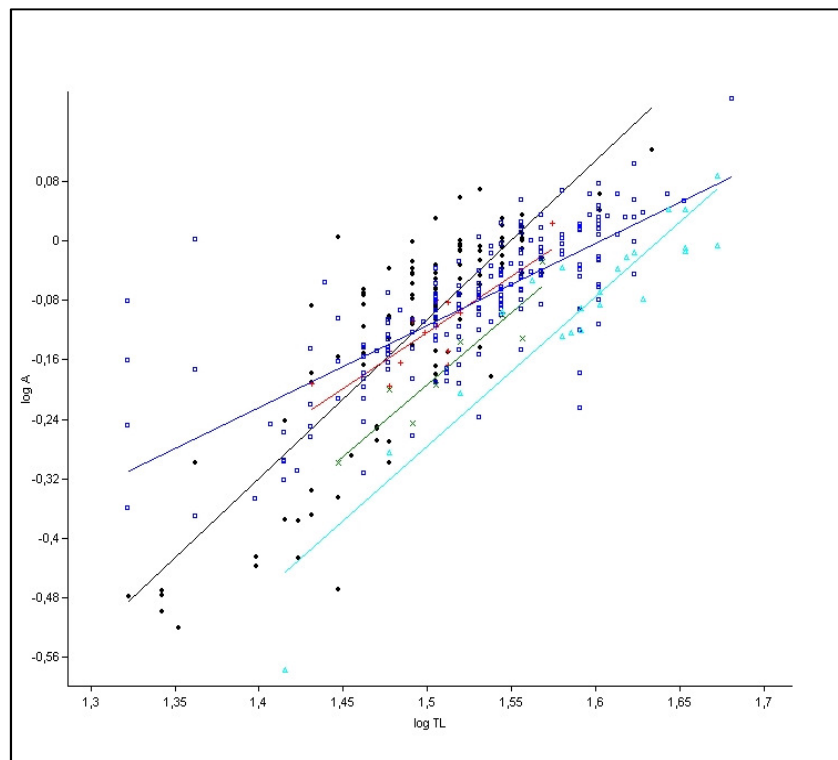
Log A – log Perim

TL – Circ

TL – Wot

TL – Feret

a2. COMPARISONS BETWEEN SEASONS

APPENDIX 18

Linear relationship between TL and Wot for the seasons of *P. microps*. Significant differences are shown in bold.

Seasons	n	Slope	Intercept	r^2	p-value
Spring	105	0,04134	-0,8878	0,75635	**2,35E-33**
Summer	40	0,03725	-0,803	0,86922	**2,25E-18**
Autumn	195	0,03351	-0,6729	0,7074	**2,13E-53**
Winter	106	0,03979	-0,7215	0,62037	**1,32E-23**

APPENDIX 19

ANCOVA table of the regression between TL and Wot compared between *P. microps'* seasons. Significant differences are shown in bold.

	SS	df	MS	F	p(same)
Adj. means	1,566	3	0,52201	56,95	**3,93E-31**
Adj. error	4,0423	441	0,00917		
Adj. total	5,6083	444			
Homogeneity of slopes					
F:	3,806				
p(equal):	**0,01028**				

Linear regression of TL and Wot for the different seasons of *P. microps*. Spring (●), summer (+), autumn (□), winter (x)

TL – Wot
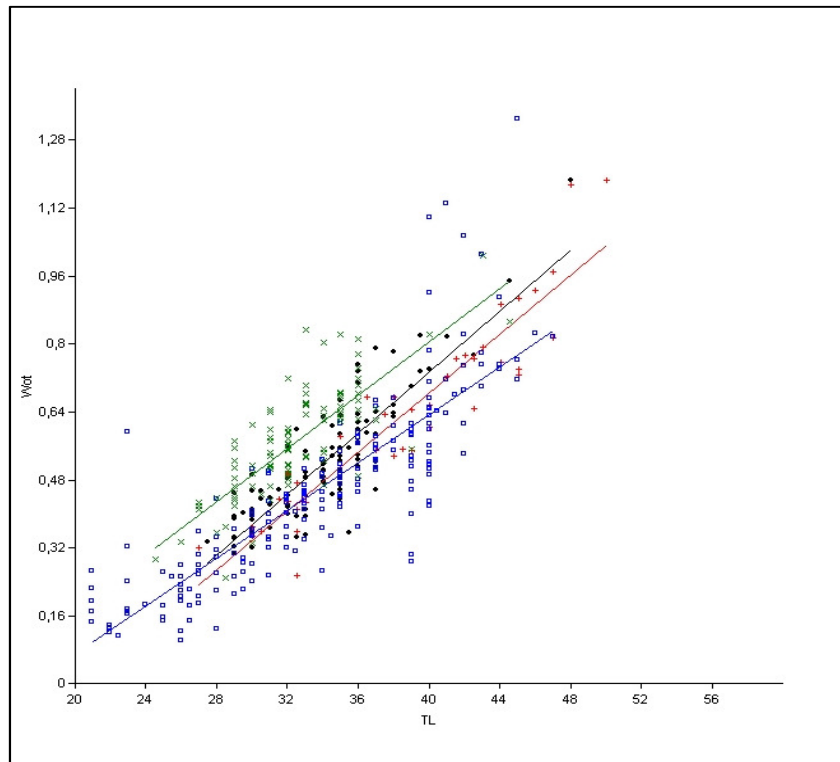
b. *Pomatoschistus minutus*

b1. COMPARISONS BETWEEN POPULATIONS

APPENDIX 21

Linear regressions for the different populations of *P. minutus*. Significant differences are shown in bold.

log TL – log A

Populations	n	Slope	Intercept	r^2	p-value
Bergen	89	2,1016	-3,3056	0,81698	**7,80E-34**
Texel	117	1,5969	-2,5479	0,58474	**1,09E-23**
Minho	18	1,5806	-2,5015	0,87608	**1,16E-08**
Valosen	28	1,9673	-3,2334	0,48532	**3,82E-05**
Trondheim	45	1,6837	-2,6991	0,91887	**4,44E-25**
Innhavet	72	1,9287	-3,144	0,85974	**1,42E-31**
Skibotn/Sørbotn	88	2,2834	-3,746	0,78255	**3,10E-30**

log A – log Perim

Population	n	Slope	Intercept	r^2	p-value
Bergen	101	0,5128	0,56107	0,99861	**2,62E-143**
Texel	117	0,57298	0,56328	0,98003	**1,42E-99**
Minho	18	0,52605	0,56505	0,99607	**1,11E-20**
Bodø	32	0,51411	0,56083	0,99968	**5,57E-54**
Trondheim	45	0,54681	0,5688	0,9933	**2,21E-48**
Innhavet	72	0,54144	0,5689	0,99619	**2,11E-86**
Skibotn/Sørbotn	90	0,50801	0,55772	0,99958	**1,81E-150**

TL – Circ

Population	n	Slope	Intercept	r^2	p-value
Bergen	89	-0,002	1,0357	0,5321	**5,19E-15**
Texel	108	-0,005	1,1451	0,18788	**5,14-6**
Minho	18	-0,0035	1,0664	0,40065	**4,87E-03**
Bodø	29	-0,0017	1,0232	0,34079	**8,86E-04**
Trondheim	45	-0,005	1,1207	0,46957	**2,07E-07**
Innhavet	72	-0,0048	1,1173	0,45212	**9,94E-11**
Skibotn/Sørbotn	89	-0,0014	1,0234	0,3398	**3,03E-09**

TL – Feret

Population	n	Slope	Intercept	r^2	p-value
Bergen	88	0,03361	-0,0524	0,82616	**2,00E-34**
Texel	117	0,02561	0,22259	0,60089	**1,10E-24**
Minho	18	0,02986	0,1016	0,79805	**6,00E-07**
Bodø	30	0,02834	-0,357	0,57129	**1,37E-06**
Trondheim	45	0,02639	0,16005	0,9169	**7,46E-25**
Innhavet	72	0,02987	-0,059	0,83819	**2,14E-29**
Skibotn/Sørbotn	89	0,03185	-0,1903	0,75352	**3,41E-28**

TL - Wot

Population	n	Slope	Intercept	r^2	p-value
Bergen	85	0,00486	-1,0749	0,37715	**4,09E-10**
Texel	117	0,03935	-0,9019	0,80399	**1,67E-42**
Minho	16	0,03806	-0,8108	0,64474	**0,00018**
Bodø	27	0,05021	-1,286	0,63366	**6,88E-07**
Trondheim	42	0,0353	-0,7632	0,78617	**5,61E-15**
Innhavet	69	0,03054	-0,7119	0,83923	**2,70E-28**
Skibotn/Sørbotn	99	0,05761	-1,6653	0,8407	**1,78E-40**

APPENDIX 22

ANCOVA table of the different regressions compared between *P. minutus*' populations.
Significant differences are shown in bold.

log TL – log A

	SS	df	MS	F	p(same)
Adj. means	0,97295	6	0,16216	22,66	**2,32E-23**
Adj. error	3,2136	449	0,00716		
Adj. total	4,1866	455			
Homogeneity of slopes					
F:	6,235				
p(equal):	**2,67E-06**				

log A – log Perim

	SS	df	MS	F	p(same)
Adj. means	0,00901	6	0,0015	43,84	**1,82E-42**
Adj. error	0,016	467	3,43E-05		
Adj. total	0,025	473			
Homogeneity of slopes					
F:	26,52				
p(equal):	**3,79E-27**				

TL – Circ

	SS	df	MS	F	p(same)
Adj. means	0,16636	6	0,02773	57,86	**1,11E-52**
Adj. error	0,21179	442	0,00048		
Adj. total	0,37815	448			
Homogeneity of slopes					
F:	7,969				
p(equal):	**3,60E-08**				

TL – Feret

	SS	df	MS	F	p(same)
Adj. means	2,2341	6	0,37236	26,6	**3,82E-27**
Adj. error	6,3124	451	0,014		
Adj. total	8,5465	457			
Homogeneity of slopes					
F:	5,034				
p(equal):	**5,22E-05**				

TL – Wot

	SS	df	MS	F	p(same)
Adj. means	0,16636	6	0,02773	57,86	**1,11E-52**
Adj. error	0,21179	442	0,00048		
Adj. total	0,37815	448			
Homogeneity of slopes					
F:	7,969				
p(equal):	**3,60E-08**				

Linear regressions for the different populations of *P. minutus*. Bergen (●), Texel (+), Minho (□), Valosen (x), Trondheim (Δ) , Innhavet (○) and Skibotn/Sørbotn (◊)
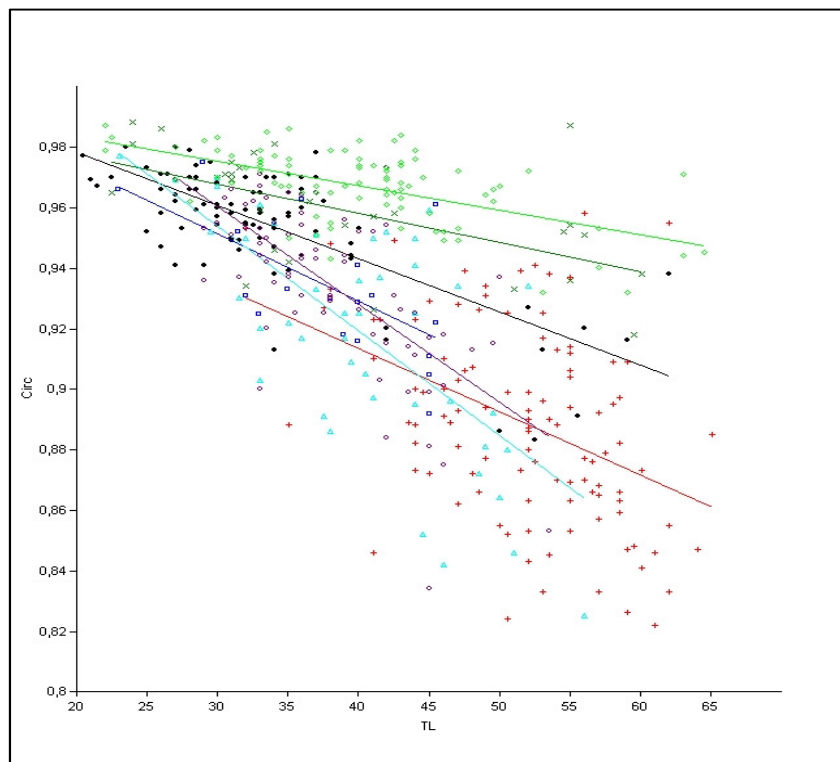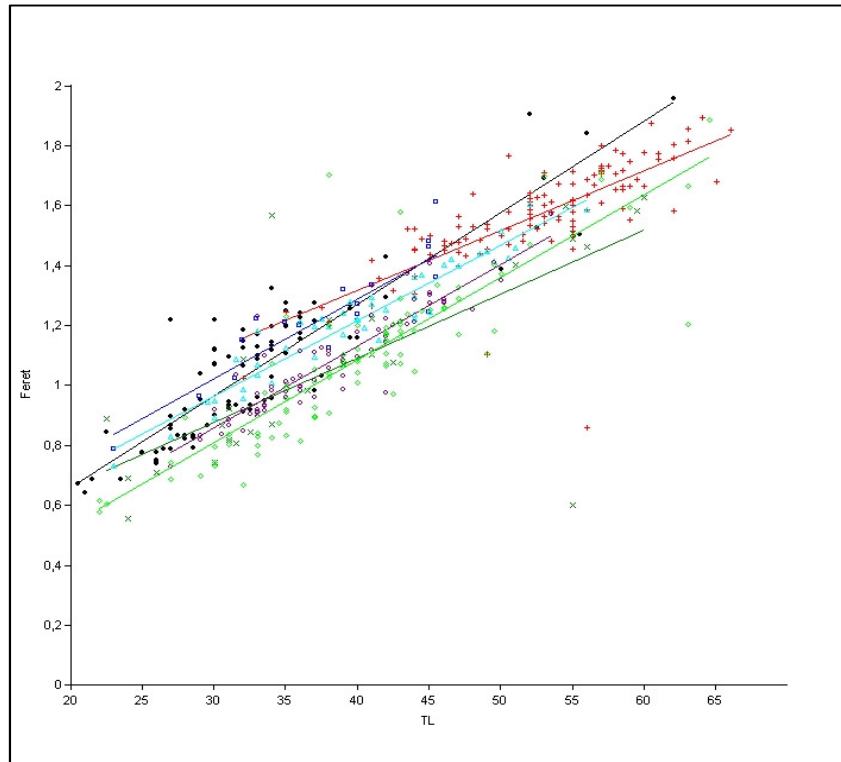
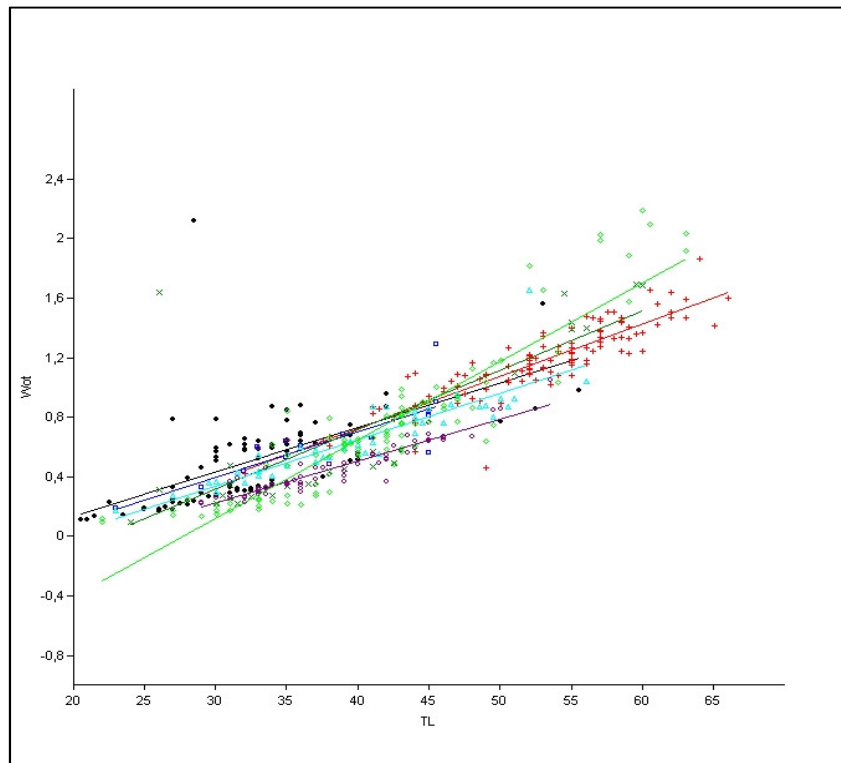Log TL – log A
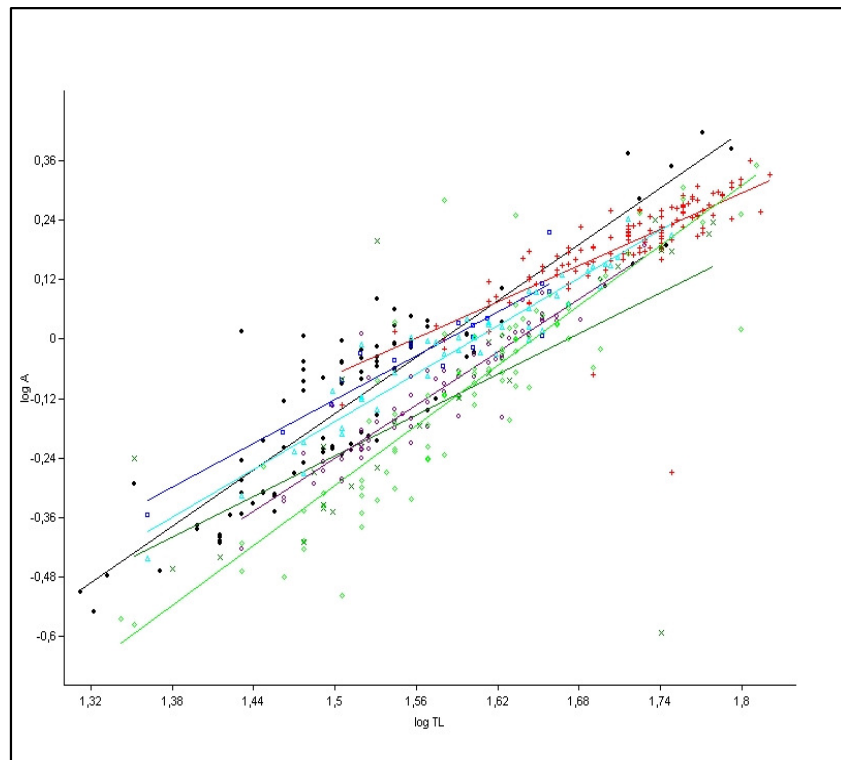
Log A – log Perim
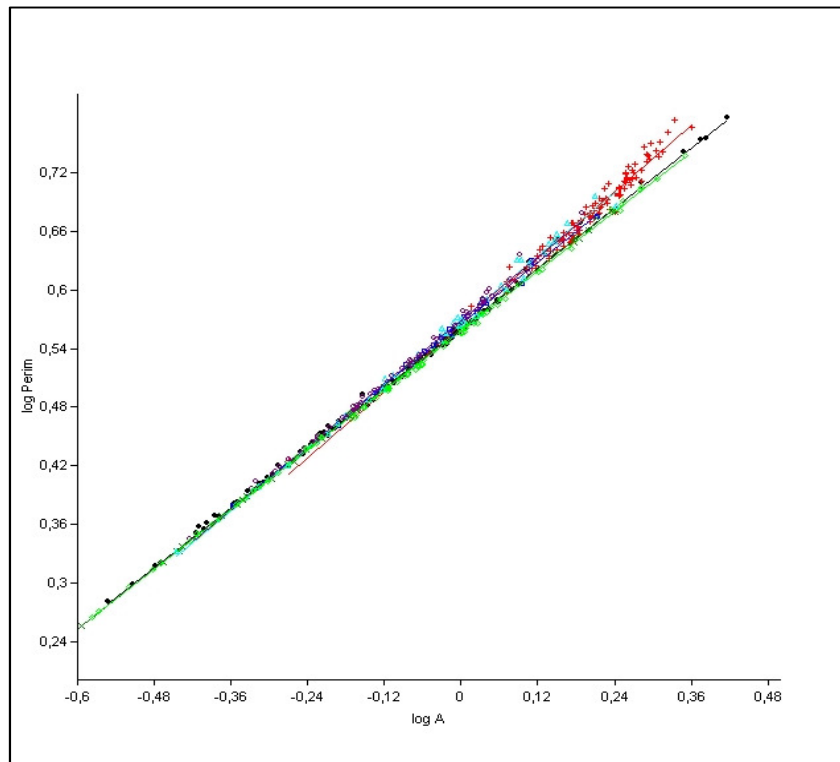
TL - Circ

TL – Feret

TL – Wot

b2. COMPARISONS BETWEEN SEASONS

APPENDIX 24

Linear relationships between TL and Wot for the seasons of *P. minutus*. Significant differences are shown in bold.

| Seasons	n	Slope	Intercept	r^2	p-value
summer	189	0,04731	-1,2781	0,77147	**7,64E-62**
autumn	221	0,04134	-1,0283	0,59663	**4,64E-45**
winter	40	0,0461	-0,8996	0,63518	**1,28E-09**

APPENDIX 25

ANCOVA table of the regression between TL and Wot compared between *P. minutus*' seasons. Significant differences are shown in bold.

	SS	df	MS	F	p(same)
Adj. means	3,183	2	1,5915	42,38	**1,41E-17**
Adj. error	16,788	447	0,03756		
Adj. total	19,971	449			
Homogeneity of slopes					
F:	8,965				
p(equal):	**0,00015**				

Linear regression of TL and Wot for the different seasons of *P. minutus.* Suumer (●), qutumn
(+) and winter (□)

TL – Wot
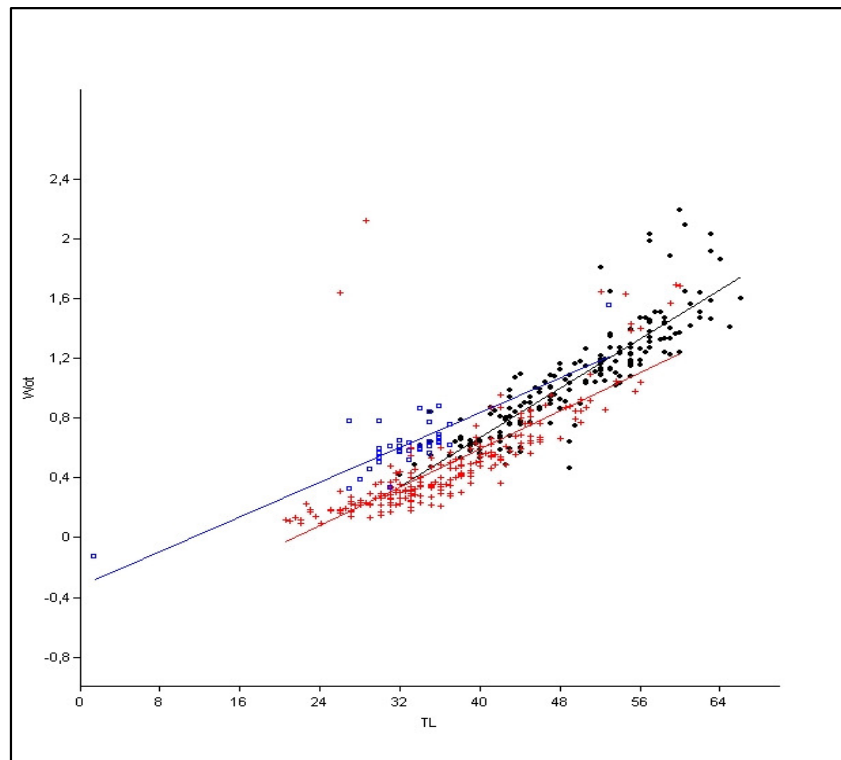
OTOLITH SHAPE ANALYSIS FOR INTER- AND INTRA-SPECIFIC DIFFERENCES

Interspecific comparisons

a. ALL POPULATIONS MERGED

APPENDIX 27

Hotelling's parwise comparisons following MANOVA for the interspecific comparisons.
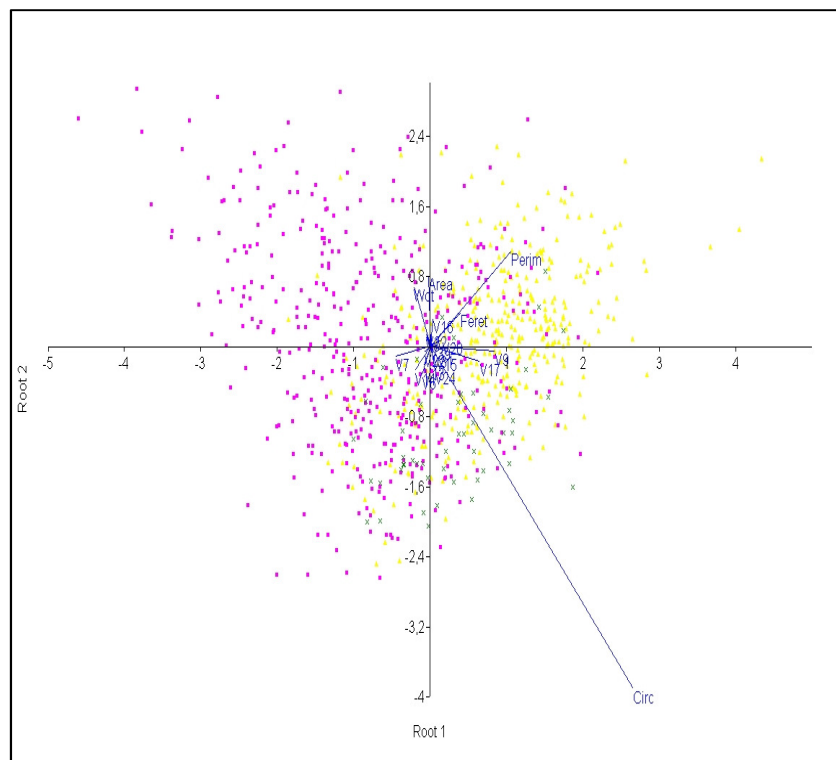Hotelling's p-values are given above the diagonal, while Bonferroni corrected values

(multiplied by the number of pairwise comparisons) are given below the diagonal. Significant differences are shown in bold.

0	P. microps	P. minutus	P. pictus
P. microps	0	**1,42E-65**	**1,46E-08**
P. minutus	**4,26E-65**	0	**1,74E-06**
P. pictus	**4,38E-08**	**5,23E-06**	0

APPENDIX 28

CVA biplot of the morphological variables and EFDs for *P. microps* (▲), *P. pictus* (x) and *P. minutus* (■)
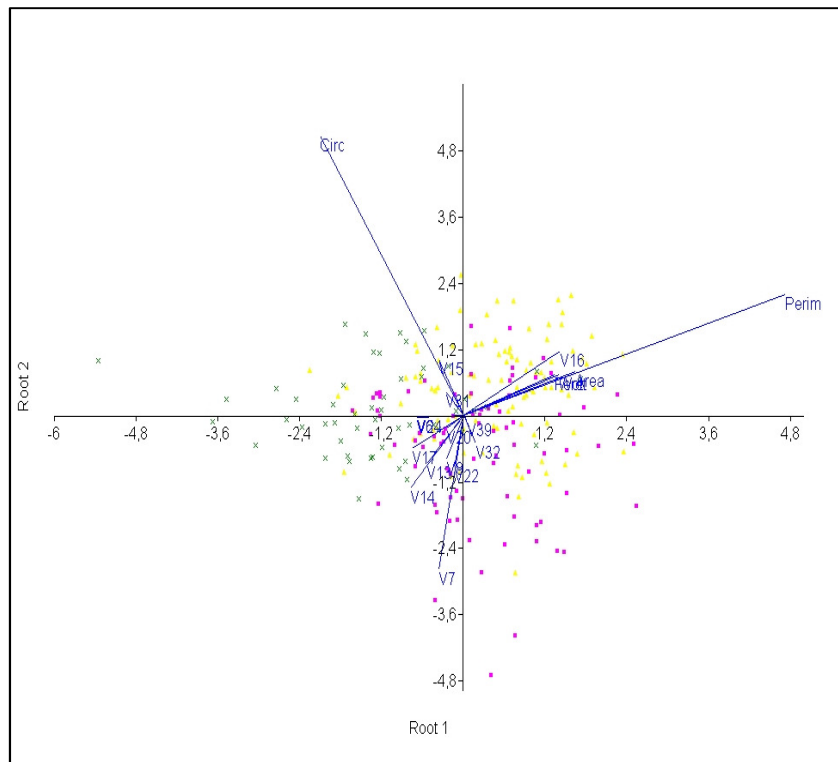

b. BERGEN

APPENDIX 29

Hotelling's parwise comparisons following MANOVA for the interspecific comparisons in Bergen. Hotelling's p-values are given above the diagonal, while Bonferroni corrected values are given below the diagonal. Significant differences are shown in bold.

0	P. microps	P. minutus	P. pictus
P. microps	0	**0,002476**	**7,57E-08**
P. minutus	0,007429	0	**7,82E-06**
P. pictus	2,27E-07	2,35E-05	0

APPENDIX 30

CVA biplot of the morphological variables and EFDs for *P. microps* (▲), *P. pictus* (x) and *P. minutus* (■) caught in Bergen

Intraspecific comparisons

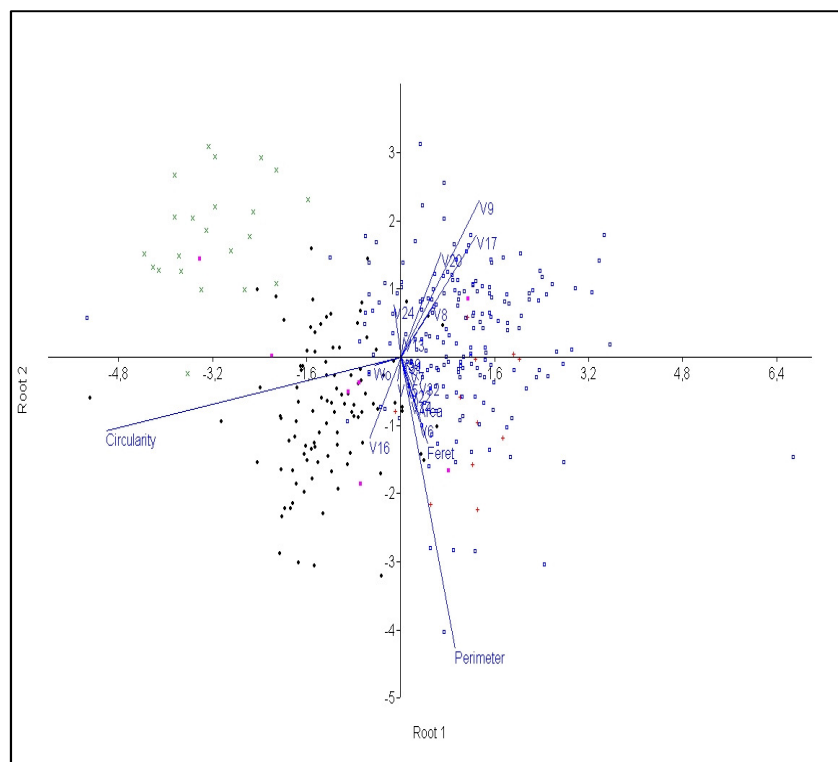### a. *Pomatoschistus microps*

a1. COMPARISONS AMONG LOCATIONS

APPENDIX 31

Hotelling's parwise comparisons following MANOVA for the comparisons between the different populations of the common goby. Hotelling's p-values are given above the diagonal, while Bonferroni corrected values are given below the diagonal. Significant differences are shown in bold.

0	Bergen	Texel	Minho	Trondheim	Skibotn/Sørbotn
Bergen	0	**3,53E-11**	**1,09E-37**	**0,03957**	**4,18E-22**
Texel	3,53E-10	0	**0,00904**	Fail	**3,38E-07**
Minho	1,09E-36	0,09045	0	**0,00013**	**3,26E-30**
Trondheim	0,39566	Fail	0,00127	0	**0,00333**
Skibotn/Sørbotn	4,18E-21	3,38E-06	3,26E-29	0,03333	0

CVA biplot of the morphological variables and EFDs for *P. microps*: Bergen (●), Texel (+), Minho (□), Trondheim (■) and Skibotn/Sørbotn (x)
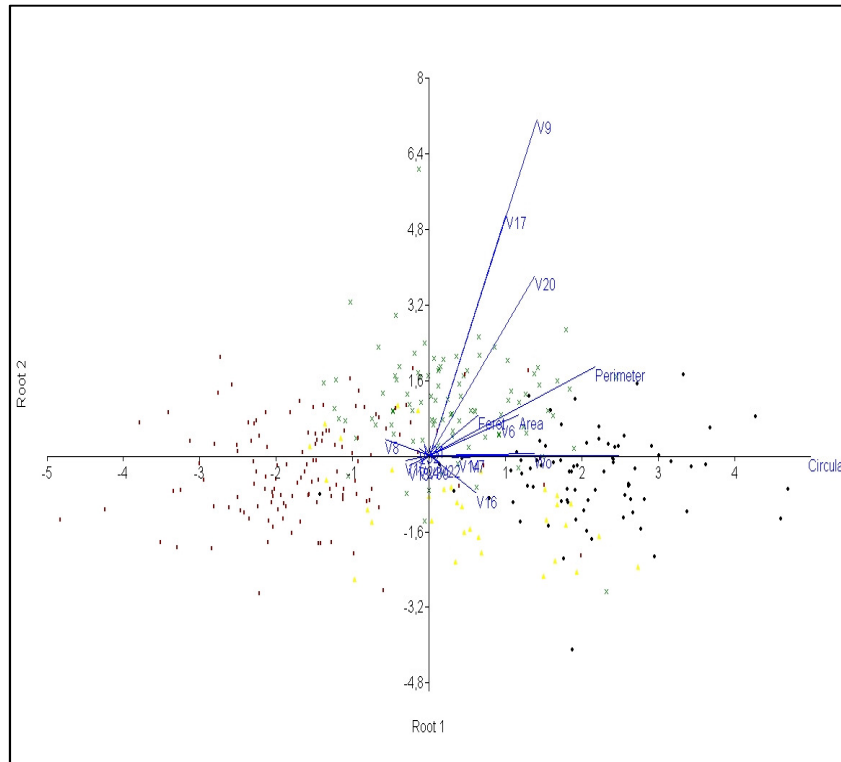

a2. COMPARISONS AMONG SEASONS

APPENDIX 33

Hotelling's parwise comparisons following MANOVA for the comparisons between the different seasons of the common goby. Hotelling's p-values are given above the diagonal, while Bonferroni corrected values are given below the diagonal. Significant differences are shown in bold.

0	spring	summer	autumn	winter
spring	0	**4,01E-11**	**5,60E-28**	**2,40E-26**
summer	2,41E-10	0	**3,97E-15**	**1,11E-12**
autumn	3,36E-27	2,38E-14	0	**2,29E-48**
winter	1,44E-25	6,68E-12	1,37E-47	0

CVA biplot of the morphological variables and EFDs for *P. microps*: spring (x), summer (▲), autumn (□) and winter (●)

b. Pomatoschistus minutus
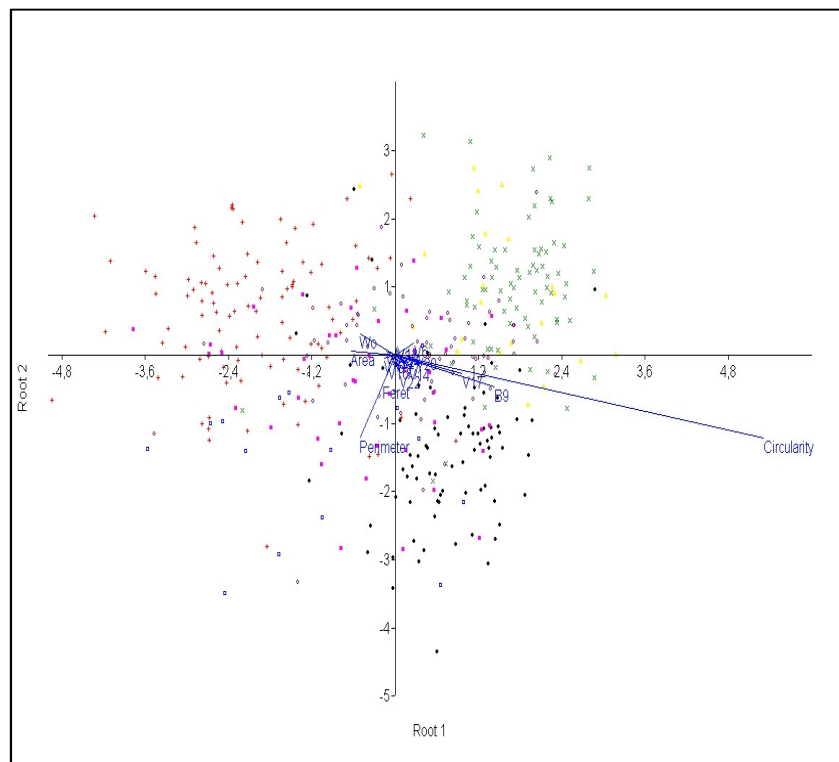
b1. COMPARISONS AMONG LOCATIONS

APPENDIX 35

Hotelling's parwise comparisons following MANOVA for the comparisons between the different populations of the csand goby. Hotelling's p-values are given above the diagonal, while Bonferroni corrected values are given below the diagonal. Significant differences are shown in bold.

0	Bergen	Texel	Minho	Valosen	Trondheim	Innhavet	Skibotn/Sørbotn
Bergen	0	**3,80E-39**	**6,94E-06**	**2,20E-12**	**3,50E-05**	**3,63E-16**	**3,51E-28**
Texel	7,98E-38	0	**8,98E-11**	**3,72E-24**	**4,38E-13**	**6,93E-41**	**3,21E-44**
Minho	0,00015	1,89E-09	0	**3,62E-05**	**0,00084**	**2,84E-09**	**3,19E-15**
Valosen	4,62E-11	7,82E-23	0,00076	0	**1,05E-07**	**1,72E-07**	**3,77E-05**
Trondheim	0,00074	9,19E-12	0,0177	2,21E-06	0	**8,67E-08**	**1,14E-18**
Innhavet	7,62E-15	1,46E-39	5,96E-08	3,62E-06	1,82E-06	0	**3,46E-20**
Skibotn/Sørbotn	7,38E-27	6,74E-43	6,69E-14	0,00079	2,40E-17	7,27E-19	0

APPENDIX 36

CVA biplot of the morphological variables and EFDs for *P. minutus*: Bergen (●), Texel (+),
Minho (□), Valosen (▲), Trondheim (■) , Innhavet (○) and Skibotn/Sørbotn (x)


b2. COMPARISONS AMONG SEASONS

APPENDIX 37

Hotelling's parwise comparisons following MANOVA for the comparisons between the
different seasons of the sand. Hotelling's p-values are given above the diagonal, while

Bonferroni corrected values are given below the diagonal. Significant differences are shown in bold.

0	summer	autumn	winter
summer	0	**8,84E-42**	**3,52E-25**
autumn	2,65E-41	0	**9,94E-36**
winter	1,06E-24	2,98E-35	0

APPENDIX 38

CVA biplot of the morphological variables and EFDs for *P. minutus*: summer (▲), autumn (□) and winter (●)
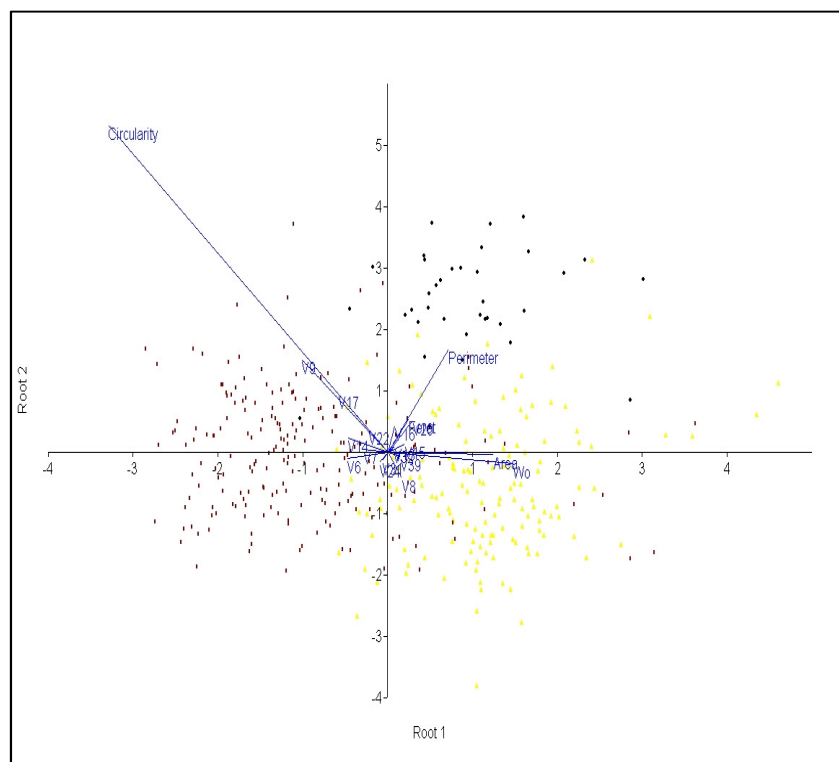
APPENDIX 39

Visual analysis of the EFDs with more impact describing shape changes. Artificial shapes represent the different possible shape changes.