



**Phenotypic and genotypic variation  
in the subtidal gastropod  
*Buccinum undatum***

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**Faculty of Life and Environmental Sciences  
University of Iceland  
2020**



# **Phenotypic and genotypic variation in the subtidal gastropod *Buccinum undatum***

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Dissertation submitted in partial fulfillment of a  
*Philosophiae Doctor* degree in Biology

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# Abstract

Variation in morphology of shelled marine gastropods across small spatial scales may reflect restricted population connectivity, resulting in evolutionary or plastic responses to environmental heterogeneity. Species delimitation of shelled gastropods is often based solely on shell characteristics; therefore, morphological variation can lead to taxonomic confusion and inaccurate estimates of species diversity. A comprehensive delimitation approach based on both phenotypic and genotypic information is needed in the face of such taxonomic uncertainty.

The common whelk *Buccinum undatum*, a subtidal gastropod ubiquitous in the North Atlantic, exhibits considerable spatial variation in shell morphology and color. The purpose of the current project was to perform a comprehensive analysis of phenotypic differentiation across the whelk's distribution and compare with a revised analysis of molecular genetic differentiation among the populations.

Phylogenetic reconstruction revealed monophyletic Eastern and Western North Atlantic whelk lineages, which diverged early in the Pleistocene glaciation (~2.1 Mya). Species screening indices indicated cryptic speciation as a result of allopatric divergence. Genetic distances between populations from the two continents were similar to or greater than interspecific genetic distances across several North Pacific and North Atlantic *Buccinum* species. Morphological differentiation in whelk populations across the North Atlantic reflected this genetic split. Concordant with observed genetic differentiation, Canadian and Icelandic whelk reared in a common garden experiment revealed consistent morphological differences between juveniles from the two continents. Finally, analysis of fine-scaled phenotypic variation of common whelk in Breiðafjörður, Iceland, revealed that shell color diversity, shape and proportion of striped individuals were all related to depth.



# Útdráttur

Svæðisbundinn útlitsbreytileiki sjávarkuðunga ber oft vitni um lítinn samgang milli stofna og getur endurspeglad þróun aðskildra stofna eða ólík vaxtarskilyrði í mismunandi umhverfi. Þar sem sjávarsniglategundir eru oft skilgreindar eingöngu út frá útlitseinkennum kuðungsins, getur slíkur útlitsbreytileiki leitt til óljósrar flokkunarfræði og ónákvæms mats á raunverulegum tegundafjölbreytileika. Það er því þörf á að skilgreina tegundir byggt á viðamiklum gögnum sem ná yfir bæði erfða- og útlitsupplýsingar.

Beitukóngur (*Buccinum undatum* L.) er algengur sjávarsnigill í Norður-Atlantshafi sem er þekktur fyrir talsverðan svæðisbundinn breytileika í lögun og lit kuðunga. Markmið þessa verkefnis var að framkvæma yfirgripsmikla greiningu á útlitsbreytileika beitukóns í Norður-Atlantshafi og bera saman við upplýsingar um hvatberabreytileika tegundarinnar.

Greining á hvatberabreytileika leiddi í ljós einættaða beitukónsstofna í austur- og vesturhluta Norður-Atlantshafs, sem hafa verið aðgreindir síðan snemma á Pleistocene jökulskeiðinu (fyrir 2,1 milljón árum síðan). Tegundaadgreiningarviðmið bentu til þess að um dultegundir (e. cryptic species) væri að ræða, sem afleiðing af sögulegum aðskilnaði (e. allopatric divergence). Erfðafjarlægðir milli stofna í heimsálfunum tveimur voru svipaðar eða meiri en erfðafjarlægðir milli nokkurra *Buccinum* tegunda í Norður-Kyrrahafi og Norður-Atlantshafi. Munur á útliti beitukóna beggja vegna Norður-Atlantshafs endurspeglar þessa erfðafræðilegu aðgreiningu. Sömuleiðis endurspegladist þessi munur í ungvíði beitukóna frá Kanada og Íslandi sem alið var upp við staðlaðar aðstæður á rannsóknarstofu. Að lokum leiddi nákvæm greining á svipgerðabreytileika beitukóns í Breiðafirði í ljós að litabreytileiki, lögun og hlutfall röndótttra einstaklinga voru háð dýpi.









*To my family*







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# List of original papers

This thesis is based on four peer-reviewed papers, three of which are published. In the text the papers are referred to with their respective numbers as follows:

- Paper I:** Magnúsdóttir H, Pálsson S, Westfall KM, Jónsson ZO and Örnólfsdóttir EB, 2018. Shell morphology and color of the subtidal whelk *Buccinum undatum* exhibit fine-scaled spatial patterns. *Ecology and Evolution* 8 (9): 4552-4563. DOI: 10.1002/ece3.4015
- Paper II:** Magnúsdóttir H, Pálsson S, Westfall KM, Jónsson ZO, Goodall J and Örnólfsdóttir EB, 2019. Revised phylogeography of the common whelk *Buccinum undatum* (Gastropoda: Buccinidae) across the North Atlantic. *The Biological Journal of the Linnean Society*, 127 (4): 890-899. DOI: doi.org/10.1093/biolinnean/blz060
- Paper III:** Magnúsdóttir H, Pálsson S, Westfall KM, Jónsson ZO and Örnólfsdóttir EB, 2019. Morphological variation in genetically divergent populations of the common whelk *Buccinum undatum* across the North Atlantic. *The Biological Journal of the Linnean Society*, 128 (1): 93-106. DOI: doi.org/10.1093/biolinnean/blz095
- Paper IV:** Magnúsdóttir H, Pálsson S, Westfall KM, Jónsson ZO and Örnólfsdóttir EB. Assessment of morphological difference between distinct lineages of common whelk (*Buccinum undatum*) in a common garden experiment (*in review at Journal of Experimental Marine Biology and Ecology*)

## Peer-reviewed papers not included in the thesis:

Goodall J, Westfall KM, Magnúsdóttir H, Pálsson S, Örnólfsdóttir EB and Jónsson ZO. RAD sequencing reveals fine scale population structuring in European Common Whelk, *Buccinum undatum*, and a genetic split across the North Atlantic (*major revision in progress, Ecol Evol*)

Jónsson ZO, Pálsson S, Westfall KM, Magnúsdóttir H, Goodall J and Örnólfsdóttir EB, 2019. The mitochondrial genome of common whelk *Buccinum undatum* (Neogastropoda: Buccinidae). *Mitochondrial DNA Part B: Resources* 4(1): 458-460. DOI: 10.1080/23802359.2018.1545534

Pálsson S, Magnúsdóttir H, Reynisdóttir S, Jónsson ZO and Örnólfsdóttir EB, 2014. Divergence and molecular variation in common whelk *Buccinum undatum* (Gastropoda: Buccinidae): a trans-Atlantic comparison. *The Biological Journal of the Linnean Society* 111:145-159. DOI: 10.1111/bij.12191





*"I expect that some of my scientific readers will lay down the book and say to themselves, 'Well ! I wonder where all this radical innovation will end! Who can possibly doubt M. elliptica being a good species ? Why, it is much smaller, of an oblong shape, thin, and glossy; while M. solida is triangular, thick, and dull. Even the young of each species exhibits its peculiar characteristics.' In reply I would ask the annotators to recollect the much greater difference that exists between shells of Buccinum undatum taken at low water and at a depth of from 70 to 80 fathoms".*

P. 418, British Conchology 2, Dr. John Gwyn Jeffreys 1860.

# 1 Introduction

Speciation, as a result of natural selection, genetic processes and geographic segregation in gene flow between populations, is a central issue in modern evolutionary biology (Butlin et al. 2008, Bird et al. 2012, Marie Curie SPECIATION Network 2012). Morphological and molecular divergence of populations in allopatry is expected to increase with time, although selection or constraints affecting certain traits or molecular variants may lead to unique patterns (Johannesson 2015, Gemmell et al. 2018). Divergence among populations in parapatry, however, can reflect previous historical divergence or be maintained by environmental factors which affect the development or evolution of phenotypic traits due to selection, which may override the homogenizing effect of gene flow among adjacent populations. Variation in environmental factors can have a clear spatial boundary or follow a gradient such as depth. Comparison of the proportion of genetic variation between groups ( $F_{ST}$ ) and its corresponding phenotypic proportion ( $P_{ST}$ ), can allow evaluation of whether morphological variation evolves in concert with molecular divergence, or whether there is stasis, convergent evolution to similar habitats, or rapid diversification of the morphological traits possibly due to positive natural selection (Merilä 1997, Sæther et al. 2007, Brommer 2011). Reduced morphological differentiation in conjunction with high genetic differentiation may indicate the existence of cryptic species (Allmon and Smith 2011) and lead to incorrect estimation of species diversity (Gemmell et al. 2018).

Marine invertebrate species with direct development have limited dispersal capabilities compared to species with a pelagic larval stage – a situation that may reduce demographic and genetic connectivity (Behrens Yamada 1987, Bell 2008), although adult dispersal capacity is also of importance (Johannesson 1988, Kyle and Boulding 2000, Marko 2004, Leis et al. 2011). The scarcity of studies of within-species diversity, geographical patterns and speciation in benthic gastropods specifically, is of particular concern since these species are often conspicuous and abundant in threatened ecosystems (e.g. Arctic and sub-Arctic) where spatial management and conservation strategies are constrained by a lack of data on population processes (Conover et al. 2006, Jones et al. 2007, Mengerink et al. 2014, Taylor and Roterman 2017, Woods and Jonasson 2017, CAFF 2018).

Shelled gastropods frequently display remarkable intra-specific variation in shell morphology across relatively small spatial scales. This geographical variation is often indicative of limited demographic or genetic connectivity and/or plastic responses to environmental heterogeneity (Trussell and Etter 2001, Iguchi et al. 2005, Mariani et al. 2012, Bourdeau et al. 2015, Magnúsdóttir et al. 2018). For example, shell morphology of aquatic gastropods is known to vary with predator prevalence and wave action (Vermeij 1982, Thomas and Himmelman 1988, Johannesson et al. 2010, Bourdeau and Johansson 2012, Pascoal et al.

2012, Kosloski et al. 2017) and shell color polymorphism has been attributed to multiple factors including both environmental and genotypic variation (Etter 1988, Kozminskiĭ et al. 2010, Kozminskii 2014, Johannesson and Butlin 2016) where determinants of selection include visual predators (Reimchen 1979, Byers 1990, Liu et al. 2009, Manríquez et al. 2009, De Bruyn and Gosselin 2014) and risk of desiccation or hypothermia (Etter 1988, Miura et al. 2007, Phifer-Rixey et al. 2008).

Species delimitation in shelled marine gastropods is largely based on shell morphology (Schander and Sundberg 2001, Wagner 2001, Allmon and Smith 2011), which can befuddle the taxonomy for the more plastic genera such as the benthic subtidal gastropods of the *Buccinum* genus (Shirai et al. 2010), that exhibit considerable phenotypic variability across small spatial scales. For reliable species delimitation, a comprehensive analysis with due regard to both genetic and phenotypic variability is important, as common environmental factors may similarly influence shell shape in multiple, unrelated lineages, sometimes regardless of genotypic variation (Thomas and Himmelman 1988, Trussell and Etter 2001, Bourdeau et al. 2015, Johannesson 2015, Kosloski et al. 2017). Furthermore, the phylogeography and genetic differentiation of *Buccinum* species have been studied to some extent in the Northwest Pacific (Amano 2004, Iguchi et al. 2004, 2007b, 2007a, Shirai et al. 2010, Zhang and Zhang 2017) and in the North Atlantic (Weetman et al. 2006, Mariani et al. 2012, Pálsson et al. 2014), but within the North Atlantic there is still a large knowledge gap regarding molecular based taxonomic studies within the genus.

The type species of the *Buccinum* genus, the common whelk, *Buccinum undatum* L. 1758, is well known for similar spatial variation in shell morphology (Jeffreys 1867a, 1867b, Ten Hadders-Tjabbes 1979, Thomas and Himmelman 1988, Kenchington and Glass 1998, Mariani et al. 2012), and life history traits (cf. Haig et al. 2015, Borsetti et al. 2020). The common whelk exhibits fine-scale genetic variation within areas/countries but across the North Atlantic distinct mitochondrial DNA lineages on the Western and Eastern side of the ocean are evident (Pálsson et al. 2014). Divergence between the two continents co-occurs with or predates the onset of the Ice Age (2.7 Mya (1.07-3.38 Mya)) and indicates cryptic speciation in allopatry (Pálsson et al. 2014). The observed fine-scaled genetic patterns are consistent with expectations for a marine species where internal fertilization, direct development, and low adult mobility contribute to limited demographic connectivity among populations also separated by distance (Weetman et al. 2006, Mariani et al. 2012, Pálsson et al. 2014).

Zooarchaeological information confirms that the common whelk was found on the Eastern US coast in the middle Pliocene (3.1–3.7 Mya) (Campbell 1993) and in the UK during the Miocene and Pliocene (Wood 1848). Colder ocean temperatures and shifting ocean levels during the Pleistocene glaciation (2.6 Mya) might have caused the separation of common whelk populations throughout the North Atlantic, resulting in allopatric divergence of the two larger clades on each side of the North Atlantic during, or even before, the onset of glaciation. Isolation may also have occurred as a consequence of colonization of southerly regions on both sides of the Atlantic, possibly enforced by colder climate, as is the case for other marine organisms, e.g. some starfish species (*Asterias rubens* and *Asterias forbesi* (Wares and Cunningham 2001)).

The later divergence between the Greenlandic and Canadian clades (~1.1 Myr) possibly represents another split during the oscillating glacial periods of the Pleistocene epoch (Pálsson et al. 2014). Alternatively, divergence between clades may have resulted from genetic differentiation within populations as a function of depth. Since the common whelk is a subtidal species, it is possible that it may have survived glacial periods at greater depths than currently found. This is further supported by studies on depth tolerance that found common whelk to be theoretically capable of surviving the combined thermal and hyperbaric conditions that characterize deep sea environments (Smith and Thatje 2012). Thus the species may have been able to diversify and adapt further to its habitat than species that re-colonized land and intertidal

zones after the Last Glacial Maximum (Hewitt 2000), e.g. in Iceland and the Faroe Islands where the ice cover extended beyond the coast line toward the shelf break (Sejrup et al. 2005, Hubbard et al. 2006, Norðdahl et al. 2008). Similarly, in the Japan Sea, fossils of *Buccinum tsubai* show that the species survived in the lower sublittoral to upper bathyal waters in the isolated sea during the late Pleistocene (Amano and Watanabe 2001).

The subject of this thesis is a comprehensive analysis of the phenotypic and genetic differentiation of the common whelk across the North Atlantic through comparison of its shell morphology with the revised mitochondrial lineages of the species and its putative allopatric speciation; in addition to the phenotypic differentiation of the species on a fine-scale within Breiðafjörður, Iceland.

## 1.1 The common whelk (*Buccinum undatum*)

The common or waved whelk (*Buccinum undatum* L. 1758) (Figure 1-1) is a subtidal neogastropod found on both sides of the North Atlantic (Golikov 1968). On the European side it is referred to as the common whelk or buckie, while on the North American side it is aptly named the waved whelk, since the literal translation of its Latin name is “Wavy Trumpet”, referring to the typical wavy texture of its shell. The common whelk can reach up to 150 mm in shell height and an age of 13 years. It usually lives just below the tidal zone down to a depth of 200 m but is also found in deeper waters (Golikov 1968, Óskarsson 1982).

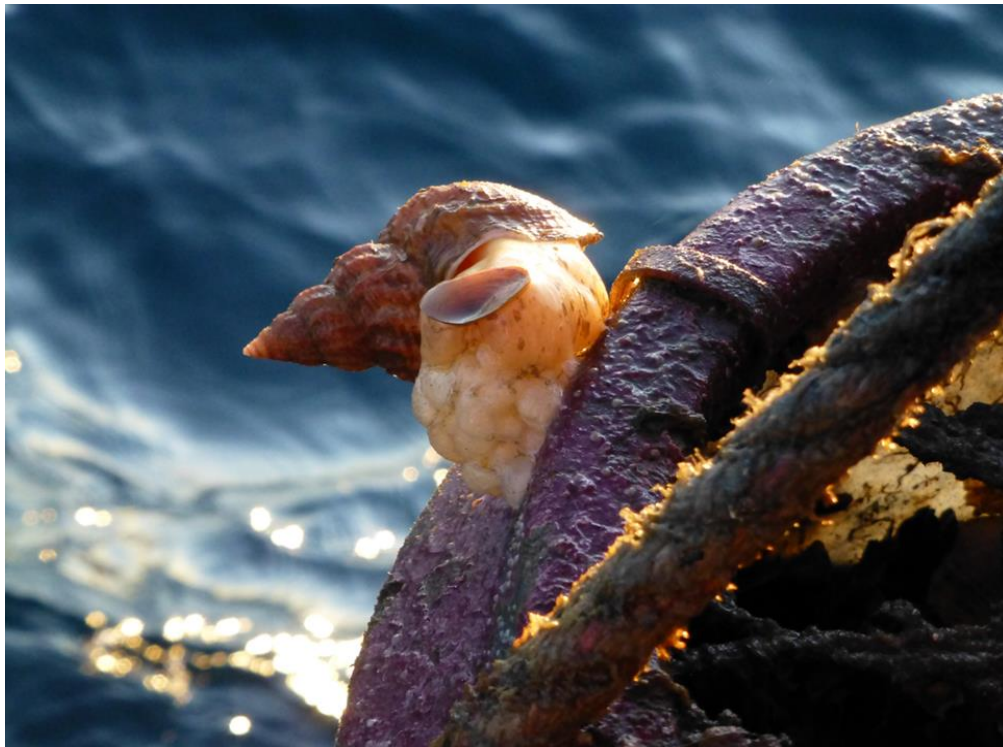


Figure 1-1. A common whelk female laying an egg-mass on a retrieved trap in Breiðafjörður.

The species has been utilized for human consumption and bait throughout the centuries in Europe. In southeast England for example, there is a long history of human consumption of whelk due to its local abundance and whelk shells have been found mixed with oyster shells in Roman kitchen middens. A record of the enthronement feast of the Bishop of Canterbury in 1500 A.D. states that 8000 whelks were included in the festivities (Fretter and Graham 1994). Fishing gear consisted mostly of wicker baskets that were put down on a muddy bottom

(Jeffreys 1867a) but whelks were also a by-catch from dredges of oyster beds (Campbell and Russell 2014). In Iceland, the common whelk was mainly used for bait which gave rise to its Icelandic name, *beitukóngur* or “bait king”. However, there are records of whelks being eaten around *Breiðafjörður*, location of the highest density of whelks in Iceland, and in *The Travel Book* of Eggert and Bjarni from 1772 whelk cooking methods from that area are described. Whelks were mostly collected by putting nets with codskin at the bottom of the intertidal zone at low tide and dragging them up when the tide came back in.

Today the common whelk is fished commercially with baited traps (Figure 1-2) in many countries in NW-Europe and North America. The most extensive fisheries are in Canada and in the sea around France, UK and Ireland, but smaller fisheries are also arising in recent years in Sweden, Norway, Faroe Islands, Iceland and the US. The biggest markets for whelks are in SE-Asia and S-Europe.



*Figure 1-2. Baited whelk traps (left) and whelk salad from Quebec (right).*

## **1.1 Life history and biology of the whelk**

### **1.1.1 Reproduction and development**

The common whelk is gonochoric (either male or female) with internal fertilization. The males have a long penis that can reach into the shell of the female and penis length is used as an indicator of sexual maturity; males are considered to be sexually mature when the length of the penis has reached half the height of the shell or more (Køie 1969). After up to eight weeks from mating, females lay masses of egg-capsules attached to a hard substrate, e.g. rocks, seagrass, seaweed (Martel et al. 1986a). An average egg-mass laid by one female contains 140 capsules, but several females often aggregate to lay eggs together in one large mass (Figure 1-3). In each capsule there can be up to 3200 whelk embryos but only about 1% complete their development; the rest serve as nurse eggs for their siblings (Martel et al. 1986a, Fretter and Graham 1994, Valentinsson 2002, Smith and Thatje 2013). The offspring exhibit direct development, i.e. there is no free-swimming planktonic larval stage, instead they go through the trochophora and veliger stages inside the egg capsule and then crawl out as tiny fully developed whelk about 2-3 mm in shell height (Martel et al. 1986b).

In Canada, mating of the common whelk takes place from mid-May to the beginning of July and egg-laying takes place from the end of May to the end of August (Martel et al. 1986a, 1986b). In Europe, however, copulation takes place from autumn until mid-winter (Kideys et



al. 1993, Valentinsson 2002, Magnúsdóttir 2010, cf. Borsetti et al. 2020). Development time differs between the continents as well; Canadian juveniles hatch from their capsules 5-8 months after egg-laying (Martel et al. 1986a) whereas in Britain hatching takes only 3-5 months (Kideys et al. 1993). Thus, the reproduction of whelk from the two continents is both spatially and temporally isolated.

The temporal displacement in mating and development between continents on either side of the North Atlantic means that recruitment of whelks occurs in autumn and winter in Canada while in Europe it takes place in spring and summer. Several hypotheses have been put forward to explain this phenomenon, Martel et al. (1986b) postulated that since the winter sea temperature in Europe is higher than the average summer temperature in the Gulf of St. Lawrence, a cold-water species such as the common whelk might find the warm summer temperatures in Europe unfavorable for its embryonic development. Furthermore, both Martel (1986b) and Laptikhovsky (2014) have suggested the temporal displacement in the Gulf of St. Lawrence from the rest of the species is a result of an adaptation to protect hatchlings from seasonal predatory pressure from their main predator the starfish *Leptasterias polaris*. This starfish species is not found in Europe and has been shown to have an intricate predator-prey relationship with the common whelk in the Gulf of St. Lawrence (Rochette and Himmelman 1996, Morissette and Himmelman 2000, Rochette et al. 2001).

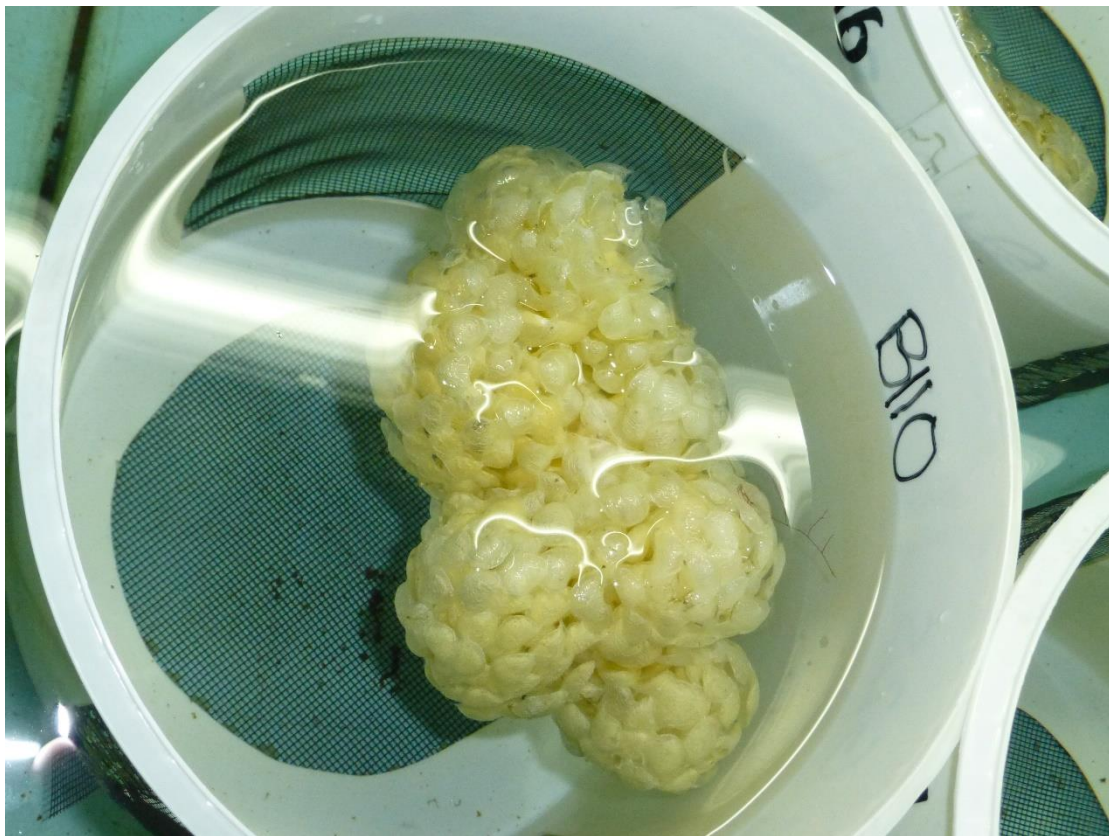


Figure 1-3. Whelk egg-mass from Breiðafjörður that was deposited by several females. Multi-maternal egg-masses are characterised by several ball-like egg-masses stuck together while an egg-mass from one female consists of a single ball. The ball-like structures consist of many egg-capsules stuck together.

### **1.1.2 Predator and prey**

As a versatile species that thrives on various bottom substrates (e.g. sand, mud, gravel or rocks), the common whelk is both an important predator and a scavenger in subtidal communities and feeds among other things on polychaetes, bivalves, echinoderms and small crustaceans (Nielsen 1975, Jalbert et al. 1989, Himmelman and Hamel 1993). The density of the common whelk is usually less than one individual per square meter, but it has been known to reach 1.8 individuals per square meter in the Gulf of St. Lawrence in Canada, where it was also the most abundant invertebrate predator (Jalbert et al. 1989). The whelk can move relatively fast, 11.4 cm/min, when alerted to possible prey, however, studies of the common whelk in Canada using diving observations found that whelk rarely actively search for food, suggesting that active predation is not the primary feeding method (Himmelman 1988, Jalbert et al. 1989, Himmelman and Hamel 1993). The species is a part of the diet of many species, including commercial ones such as cod (*Gadhus morhua*), wolffish (*Anarhicas lupus*), common eider (*Somateria mollissima*), Atlantic rock crab (*Cancer irroratus*), European green crab (*Carcinus maenas*) and great spider crab (*Hyas araneus*) (Thomas and Himmelman 1988, Kristjánsson et al. 2013, Magnúsdóttir et al. 2018) making it clear that the common whelk has an important position in the food web throughout its distribution.

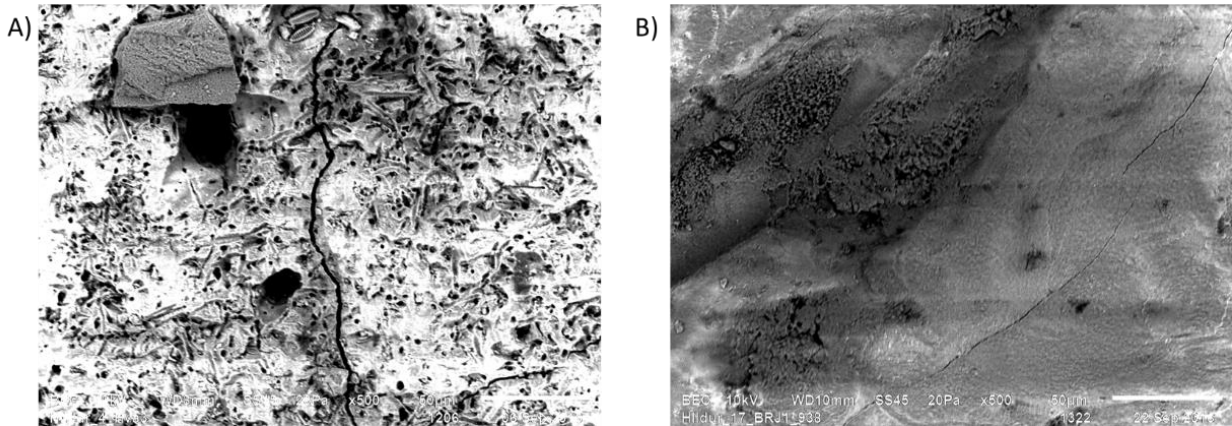
## **1.2 Morphological variation in the common whelk**

### **1.2.1 Shell shape**

Together with other *Buccinum* species (Golikov 1968), the common whelk exhibits similar spatial variation in shell morphology across its distribution (Jeffreys 1867a, 1867b, Ten Hallers-Tjabbes 1979, Thomas and Himmelman 1988, Kenchington and Glass 1998, Mariani et al. 2012), even over relatively short distances (~20 km ;Magnúsdóttir 2010). Early malacologists such as Jeffreys (1867b) noted the difference between common whelk from shallow and deep areas. A synthesis on the relation of distribution and variability of long-lived benthic animals to currents and hydrology detailed how the various morphological forms of the common whelk reflected the hydrological conditions of their habitat (Golikov 1968). In two separate studies by Ten Hallers-Tjabbes (1979) and Kenchington and Glass (1998), the whelk exhibited sexual dimorphism; females were significantly higher and had heavier shells than males, which could be a result of selection for increased female fecundity, which has been shown to be positively correlated with shell size in the whelk as in other gastropod species (Valentinsson 2002). Thomas and Himmelman (1988) linked increased shell thickness and elongated apertures of Canadian whelks with lobster and crab predation in the Gulf of St. Lawrence while thinner shells reflected negligible crustacean predation. Furthermore, differentiation in shell shape and thickness of whelks around Ireland appears to be driven by environmental variation (Mariani et al. 2012).

## 1.2.2 Shell color

The common whelk exhibits significant phenotypic variation with regards to color, which can be sorted into two categories: 1) External or environmental coloration due to epibionts and 2) Internal color variation, determined by pigments secreted by the mantle in formation of the shell.



*Figure 1-4. SEM images of the surface of the shell of the common whelk, both images are magnified x500. A) Depicts the surface of a green shell. B) Depicts the surface of a white shell (images by Hildur Magnúsdóttir).*

Green and rosy coloration of the shell, through which other coloration/stripes can sometimes be discerned, have been observed throughout the whelk's distribution (Magnúsdóttir pers.obs., Hollyman 2017). This color has been hypothesized to stem from epibionts residing in the shell, such as algae or cyanobacteria for the green color (Hollyman 2017) and coralline algae for the rosy color (Gunnarsson and Einarsson 2000). Indeed, detailed scrutiny by Hollyman (2017) of shell sections using SEM revealed hundreds of small holes in green whelk shells from Shetland, extending from the surface of the shell into the inner nacreous layer, which were not found in shells with other coloration and the nature of the holes indicated that they had been caused by microalgae or sponges. Similarly, SEM of green whelks from Breiðafjörður in the present study revealed a very porous shell surface compared to white whelks (Figure 1-4). Treatment of the Shetland shells by NaOH further supports the relationship with microalgae or cyanobacteria, since this leached the green color out of the shells (Hollyman 2017), indicating that the chlorophyll in the algae was being drawn out as the algal cells broke down.

Aside from the aforementioned green and rosy coloring, the common whelk still exhibits extensive color polymorphism across its distribution. To date, the most variable color in the common whelk is found in Breiðafjörður, West Iceland. This could however be biased due to the almost 13 years of intensive studies of whelk morphology in the area as both anecdotal information and personal communications indicate extensive color variation in other countries as well. Whelk in Breiðafjörður are found in many shades of red, white, yellow, orange, brown, beige, purple and blue, often with spiral or longitudinal stripes. The spire can be in a different shade of color to the body whorl, probably due to the whelk's long lifespan (up to 13 years) and weathering of the shell, or varying food sources. Additionally, shell break repairs are often in a different color than the original shell.

## 1.3 Dispersal and genetic patterns

The widespread distribution of the common whelk in the N-Atlantic would seem to contradict the prediction of limited distribution of marine species without a pelagic larval stage (Behrens Yamada 1987, Bell 2008). However, studies have found several instances where direct developing gastropods have either colonized new areas before species with a pelagic larval stage (Johannesson 1988, Johannesson et al. 1995) or where depleted areas have quickly regained their genetic diversity (Colson and Hughes 2004). Rafting of individuals on seaweed is one possible explanation for these contradictions (Johannesson 1988, Ingólfsson 1992, 1995, 1998, Marko 2004, Thiel and Hays 2006). However, since the common whelk is a benthic species, it is more likely that egg-masses come loose from the substrate to which the females have attached them, and drift with the currents (Donald et al. 2015). Viable egg-masses have been collected on the beaches in Breiðafjörður and reared for hatching by Smith and Thatje (2012). The main benefits for species with individuals hatching from drifting egg-masses would be that in isolated locations they are more likely to have access to a selection of mates than species with drifting individual larvae or rafting individuals. Additionally, since whelk egg-masses are often both multipaternal and multimaternal (Martel et al. 1986a), founder effects in such cases would be reduced. Nevertheless, since the distribution of whelk with drifting egg-masses has not been studied, it must be assumed that in general, colonization of new areas takes place with population boundaries expanding slowly over time and with climatic cycles, as indicated by direct development of juveniles and relatively sedentary adults (Himmelman 1988, Jalbert et al. 1989, Himmelman and Hamel 1993).

Phylogeographic analysis of the common whelk has brought to light clear population structure based on microsatellite, mitochondrial (COI and 16S ribosomal RNA) and RADseq variation on both small and large geographical scales (Goodall et al. submitted, Weetman et al. 2006, Mariani et al. 2012, Pálsson et al. 2014). Within Europe, divergence follows the isolation-by-distance model, where small but significant differentiation is observed between countries, characteristic of populations with limited demographic connectivity, and even within countries, e.g. around the UK and along the coast of Iceland (Goodall et al. submitted, Weetman et al. 2006, Mariani et al. 2012, Pálsson et al. 2014). Distinct mitochondrial DNA (mtDNA) lineages in the Western and Eastern North Atlantic Ocean indicate that populations from the two continents diverged close to the onset of the last Ice Age (~2.7 Mya) and likely constitute cryptic species (Pálsson et al. 2014). Additionally, populations from Greenland and Canada have been isolated for ~1.1 Myr.

## 1.4 Aims of the thesis

The objective of this thesis can be divided into three main goals. Firstly, to quantify and compare the morphological characteristics of the common whelk, both within Iceland and across the North Atlantic. Secondly, to revise our knowledge of the population structure of the common whelk across the North Atlantic and compare the population differentiation to the phenotypic variation. And thirdly, to investigate further the hypothesis of cryptic speciation between European and North American common whelk. In **Paper I**, shell shape and color of the common whelk in Breiðafjörður were studied with regards to environmental variation. In **Paper II**, the population structure and species status of the common whelk was assessed through analysis of genetic variation. In **Paper III**, differentiation in shell shape across the North Atlantic was compared with genetic differentiation and in **Paper IV** the shell shape of juvenile whelk from Canada and Iceland reared in a common garden experiment was compared in order to determine if they retain the distinct shell characteristics of their parent populations, as might be expected if they are genetically determined.



## 2 Methods

### 2.1 Sampling

Shell samples of adult common whelk were acquired from Canada, Iceland, the Faroe Islands, Greenland, and the United Kingdom (Figure 2-1). Sampling took place at a range of depths from 10 to 367 m, either with deployment of whelk traps or with dredging, over the period from 2008 to 2015 (**Paper I&III**).

Variation (**Paper II&III**) in microsatellites and the shorter mtDNA *COI* sequences (369bp, subsequently referred to as *COI-1*) was obtained from whelk sampled from 2008 to 2010 in Canada, UK, The Faroe Islands, Iceland and Greenland (Pálsson et al. 2014) while the longer mtDNA *COI* sequences (695bp, subsequently referred to as *COI-2*) were acquired from Icelandic whelk transcriptomes (Jónsson et al. 2019) and GenBank sequences from the US, Canada, UK, North Sea and Scandinavia. Homologous *COI* sequences from species within the *Buccinum* genus (**Paper II**) were obtained from GenBank and BOLD.

Egg-masses (**Paper IV**) from Iceland were collected in Breiðafjörður while Canadian egg-masses came from whelks from the Gulf of Saint Lawrence that laid egg-masses in a laboratory.

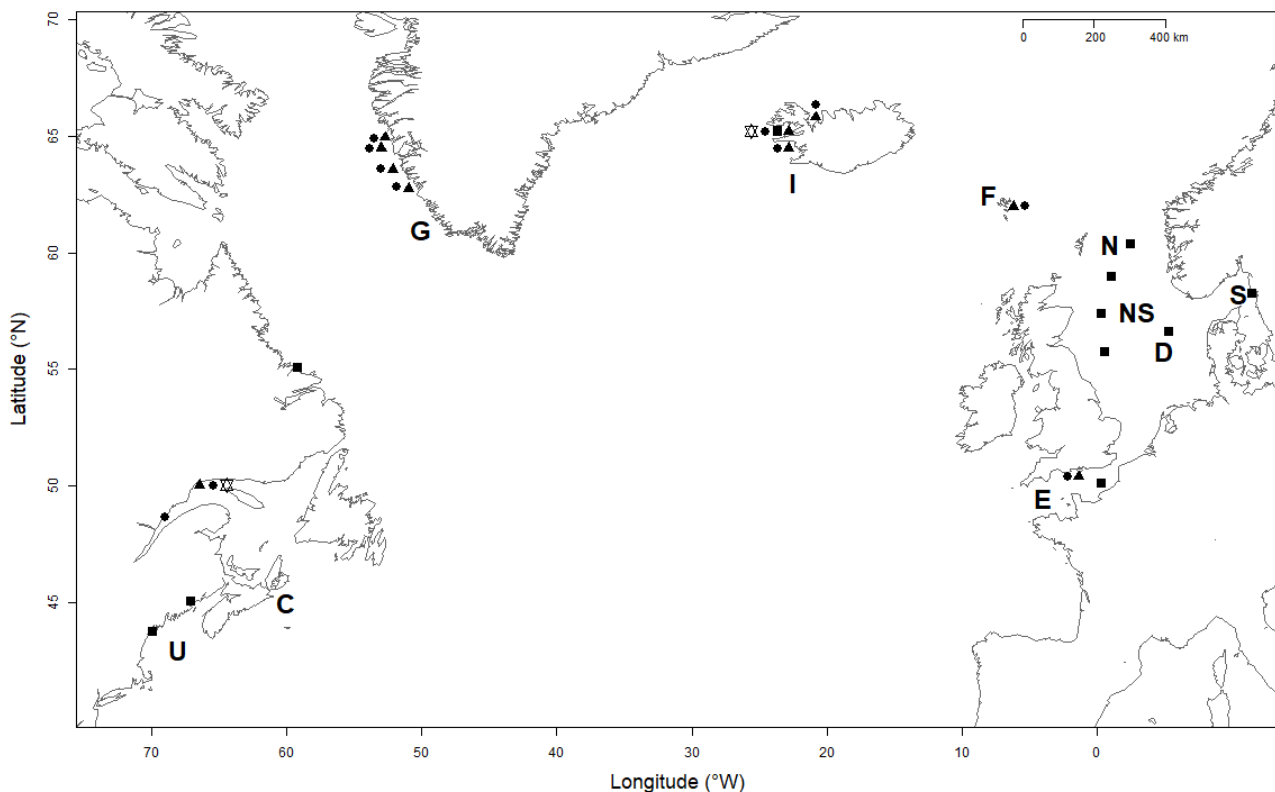


Figure 2-1. Common whelk sample sites in the North Atlantic. Areas are denoted as follows: C, Canada; D, Denmark; E, England; F, The Faroe Islands; G, Greenland; I, Iceland; NS, North Sea; N, Norway; S, Sweden; U, USA. Triangles indicate *COI-1* sequences (369 bp), squares indicate *COI-2* sequences (695 bp), circles indicate shell samples and the star indicates egg-mass samples.

## 2.2 Shell shape analysis

Three different methods were utilized to quantify the shell shape of the common whelk: traditional morphometrics (**Paper I&III**), landmark-based geometric morphometrics (**Paper I**) and shell outline analysis (**Paper III&IV**). Geometric morphometrics and shell outline analysis are more recently developed methods that are considered to offer higher resolution than traditional morphometrics (Brönmark et al. 2011, Magnúsdóttir et al. 2018) since they use a larger number of independent variables to evaluate patterns of shape variation within and between populations (Rohlf and Marcus 1993, Stransky 2005). Traditional morphometrics are however still useful for broadly delineating gastropod populations based on shell characteristics (Thomas and Himmelman 1988, Hollyman 2017, Woods and Jonasson 2017, Magnúsdóttir et al. 2018). Additionally, in large studies where shell shape information over many years from several countries is being combined, they are both easily collected with tried and tested methods (Thomas and Himmelman 1988) and shareable. Furthermore, comparing results from old and new methods provides an opportunity to estimate the usefulness and resolution of the different methods (Stone 1998). Accordingly, comparison of the methods' ability to classify the individuals to their sampling locations was done based on Linear Discriminant Analysis (LDA). The shape outline analysis was found to be the most successful method (Table 2-1) with between 90-100% of individuals being correctly sorted. The reliability of shape outline analysis might be related to the fact that the process is relatively automated which diminishes the sampling error and accounts for the entire shell outline.

*Table 2-1. Sorting predictions of individuals into groups based on Linear Discriminant Analysis of the data for all three morphological methods. Percentages in parentheses indicate the percentage of correctly sorted individuals for that site. The sites included here are from Breiðaffjörður, Iceland, see Figures 2-1 and 3-1. In **papers II** and **III** these sites are denoted as I1, I3, I4, and I5, while in **paper I** they are denoted as B1, HR, OD, and ST.*

| Shape outline             | Predict  |          |          |           |
|---------------------------|----------|----------|----------|-----------|
| True                      | I1 (B1)  | I3 (HR)  | I4 (OD)  | I5 (ST)   |
| I1 (B1)                   | 25 (93%) | 0        | 2        | 0         |
| I3 (HR)                   | 0        | 27 (90%) | 2        | 1         |
| I4 (OD)                   | 1        | 2        | 27 (90%) | 0         |
| I5 (ST)                   | 0        | 0        | 0        | 23 (100%) |
| Traditional morphometrics | Predict  |          |          |           |
| True                      | I1 (B1)  | I3 (HR)  | I4 (OD)  | I5 (ST)   |
| I1 (B1)                   | 0 (0%)   | 30       | 10       | 1         |
| I3 (HR)                   | 2        | 35 (76%) | 7        | 2         |
| I4 (OD)                   | 5        | 3        | 43 (84%) | 0         |
| I5 (ST)                   | 0        | 9        | 0        | 17 (65%)  |
| Geometric morphometrics   | Predict  |          |          |           |
| True                      | I1 (B1)  | I3 (HR)  | I4 (OD)  | I5 (ST)   |
| I1 (B1)                   | 9 (90%)  | 1        | 0        | 0         |
| I3 (HR)                   | 1        | 15 (83%) | 1        | 1         |
| I4 (OD)                   | 0        | 4        | 14 (78%) | 0         |
| I5 (ST)                   | 1        | 1        | 0        | 20 (91%)  |

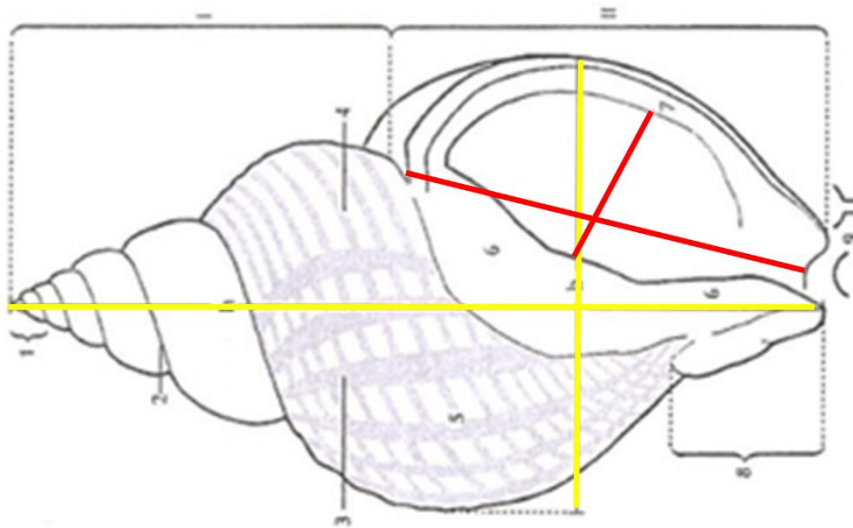


Figure 2-2. Traditional morphometric measurements of the common whelk. Yellow lines indicate shell height and width, whereas red lines indicate aperture height and width (image adapted from *Danske Havsnegle, Natur og Museum* 33:2, 1994 by Poul Bondesen).

### 2.2.1 Traditional morphometrics

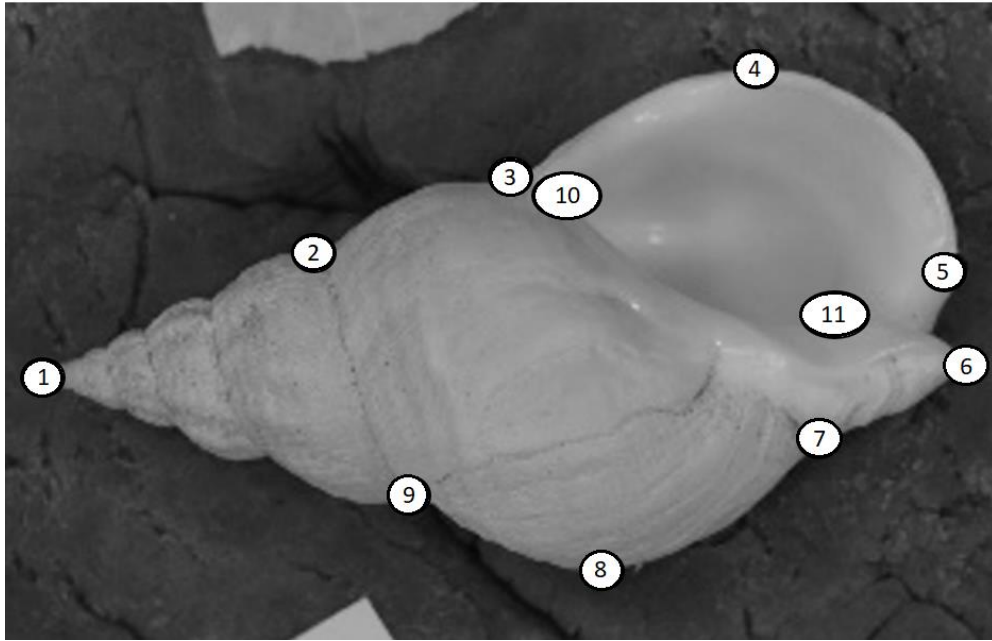
The ratio between shell height and shell width (**Paper I&III**) was used as an indicator of shell shape (Figure 2-2), i.e. round vs. elongate shells while the ratio between aperture height and width was similarly used to indicate aperture shape. Shell thickness was estimated by the ratio of the square root of the shell weight to shell height. Variation between sites was tested with ANOVA and *post hoc* Tukey's HSD.

### 2.2.2 Geometric morphometrics

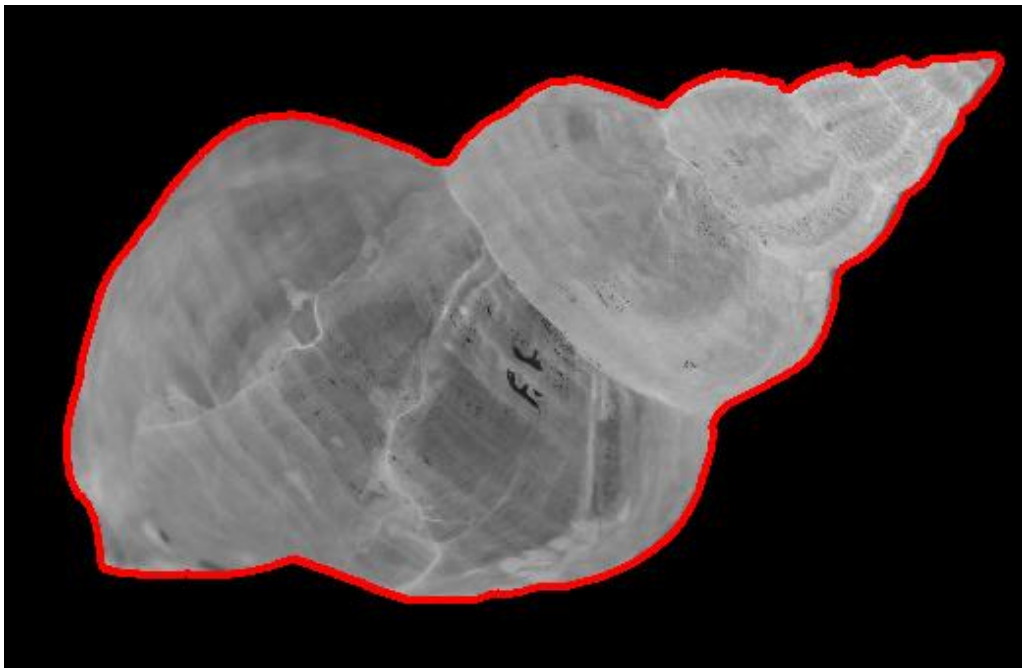
In total eleven landmarks (**Paper I**) were digitized on photographs of the ventral surface of the shells (Figure 2-3) as in Mariani et al. (2012), using the R-package *geomorph* (Adams and Otárola-Castillo 2013). Generalized Procrustes analysis and principal components analysis were subsequently performed using the same package. Shape variation between sites was analyzed with Procrustes ANOVA considering sex, longitude, depth, substrate type and proportion of decollated shells as covariates.

### 2.2.3 Outline analysis

Outline analysis (**Paper III&IV**) was conducted on the dorsal surface of the shells (Figure 2-4), photographed on a level surface. Outlines and reciprocal Wavelet coefficients were generated for the shells using the R package *ShapeR* (Libungan and Pálsson 2015). Analysis of the variation in shape between populations was done using canonical analysis of principal coordinates (CAP) in *vegan* (Oksanen et al. 2018) and significance was tested using an ANOVA-like permutation test (*anova.cca*).



*Figure 2-3. The eleven landmarks from Mariani et al. (2012) digitized in the geometric morphometric analysis of shell shape of the common whelk in Breiðafjörður, Iceland.*



*Figure 2-4. Outline of the shell of an adult common whelk collected with the R package ShapeR.*

## 2.3 Shell color analysis

Shell color of adult (Figure 2-5, **Paper I**) and juvenile common whelk (Figure 2-6, **Paper IV**) was scored manually using a Munsell-based color scale, Color Checker Classic from X-rite (<http://xritephoto.com/colorchecker-classic>). Presence and color of spiral stripes were also noted. Differences in frequency of color and stripes between sites were tested with Fisher's exact test.



Figure 2-5. A small example of the color variation in the common whelk from Breiðafjörður, Iceland (young adults and adults).

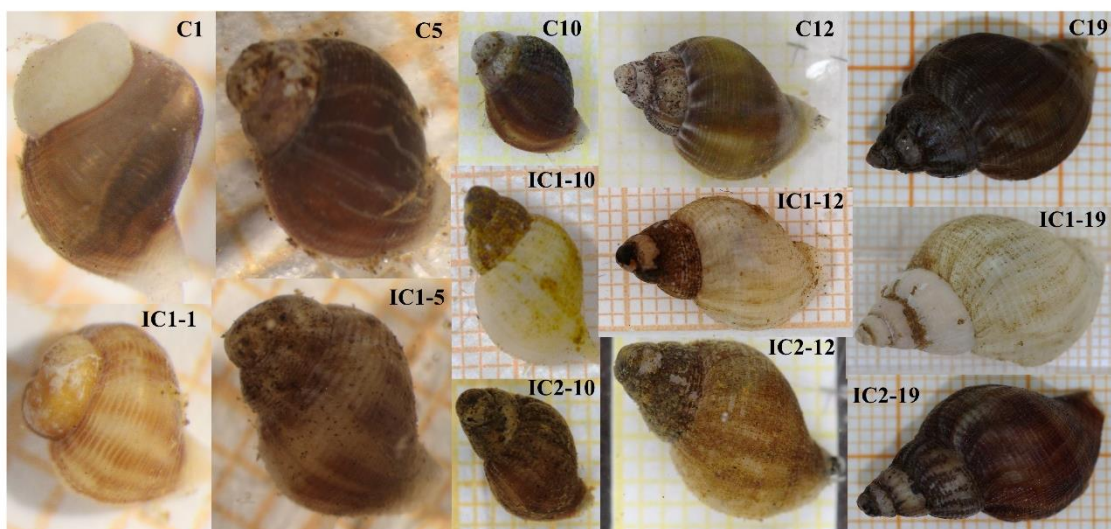


Figure 2-6. Common whelk at the age of 1, 5, 10, 12 and 19 months. Sample sites are denoted by C, Canada; IC1, Stykkishólmur, Iceland; IC2, Southeast Breiðafjörður, Iceland. The whelks are all photographed on 1 mm<sup>2</sup> paper.



## 2.4 Genetic analysis

### 2.4.1 Mitochondrial diversity

Two phylogenetic trees based on *COI* mitochondrial DNA sequences (**Paper II**) in the common whelk across the North Atlantic were reconstructed. A phylogenetic tree of the *COI-1* was calculated in BEAST (Suchard et al. 2018) and the second tree, based on the *COI-2* sequences, using PhyML in SeaView (Gouy et al. 2010) with *Buccinum pemphigus* Dall, 1907 as an outgroup. To estimate the pairwise genetic differentiation between samples, the proportion of variation between samples was calculated for both haplotype frequencies ( $F_{ST}$ ) and genetic distances between sequences ( $\Phi_{ST}$ ). Lastly, to explore the relationship of the common whelk with other species in the *Buccinum* genus, average genetic distance ( $K2P$ ) between the three main *COI* North Atlantic lineages (Europe, Greenland and Canada) were calculated for *COI-1* in the R package *ape* (Paradis and Schliep 2019) and compared with corresponding distances between 17 other *Buccinum* species from the North Atlantic and North Pacific.

### 2.4.2 Species indices

To further investigate the reliability of the geographical groupings and putative species status suggested by the phylogenetic analyses of *COI-1*, two different species status assessment methods based on the DNA barcoding gap (Hebert et al. 2003, 2004) were applied (**Paper II**); the species screening threshold index (SSTI; Witt et al. 2006) and the automatic barcode gap discovery (ABGD; Puillandre et al. 2012a). The SSTI is a conservative threshold for provisional species recognition that has been proposed at 10x the average intrapopulation *COI* haplotype divergence (Witt et al. 2006) while the more recent method of ABGD is an automatic procedure found online (Puillandre et al. 2012b) which analyses the distribution of pairwise distances among aligned sequences in order to detect a break between intra- and interspecific values to assign the sequences to putative species (Puillandre et al. 2012a).

## 2.5 Comparing phenotypic and genotypic divergence across populations ( $P_{ST}$ vs. $F_{ST}$ )

Phenotypic variance ( $P_{ST}$ ) of whelk shell shape (**Paper III&IV**) was estimated as a substitute for the additive genetic variance ( $Q_{ST}$ ), which is necessary since certain life history traits of the common whelk (late maturity and internal fertilization e.g.) make it a less than ideal candidate for the detailed crossing experiments needed to estimate quantitative genetic components. Following Brommer's (2011) formulation we incorporated the uncertainties of heritability ( $h^2$ ) and the between-population additive genetic component ( $c$ ):

$$P_{ST} = \frac{\frac{c}{h^2} \sigma_B^2}{\frac{c}{h^2} \sigma_B^2 + 2\sigma_W^2}$$

where  $\sigma_B^2$  and  $\sigma_W^2$  are phenotypic variances between and within populations, respectively. When  $c/h^2 = 1$ ,  $P_{ST}$  equals  $Q_{ST}$ , and when  $c/h^2 = 2$  it equals the intra-class correlation. Variances were based on the average of squared distances,  $d$ , between individuals  $i$  and  $j$  as  $s^2 = \sum d_{ij}^2 / N_i N_j$ , where  $N$  is the sample size. The variance components were summarized from the output from pairwise comparisons with the *adonis* function in the R package *vegan* (Oksanen et al. 2018).  $F_{ST}$  was estimated based on Weir and Cockerham (1984).

Varying environmental conditions affecting different whelk populations can create an inequality of  $c$  and  $h^2$  (Sæther et al. 2007, Pujol et al. 2008, Brommer 2011, Magnúsdóttir et al. 2018). Therefore the sensitivity of  $P_{ST}$  comparisons (**Paper III&IV**) with the neutral expectation of genetic drift and migration, was evaluated by using a selection of simulated values of  $c/h^2$  (from 0.2 to 2.0, as in Brommer (2011)).

## 2.6 Common garden experiment

A common garden experiment was designed in order to study the interaction of environmental and genetic effects on the shell morphology of the common whelk (**Paper IV**), both on a fine- (within Breiðafjörður) and large-scale (across the North Atlantic). Common garden experiments allow us to control for possible environmental effects on morphological variation in animal population (Pascoal et al. 2012, Villemereuil et al. 2015) by rearing individuals from the same species or population from the egg/larval stage in a controlled environment and estimate genetic components and partition of genetic variance among groups (Sæther et al. 2007, Pujol et al. 2008, Brommer 2011, Villemereuil et al. 2015). If the genetic effect on the morphological characteristics is independent of the environment, individuals from different areas will maintain their distinct morphology during the experiment, whereas if the characteristics are the result of plastic responses to the environment, the organisms will have similar genetic components and partition of genetic variance among groups (Sæther et al. 2007, Pujol et al. 2008, Brommer 2011, Villemereuil et al. 2015). Egg-masses and juveniles (after hatching) were reared in 4-liter buckets with aerated seawater at 3-6° C (Smith and Thatje 2012) at Hólar University and cohorts of potential siblings (i.e. juveniles from a single egg-mass) were reared in separate buckets (Fig 2-7) and shell morphology and color quantified at 1, 5, 10, 12 and 19 months after hatching.



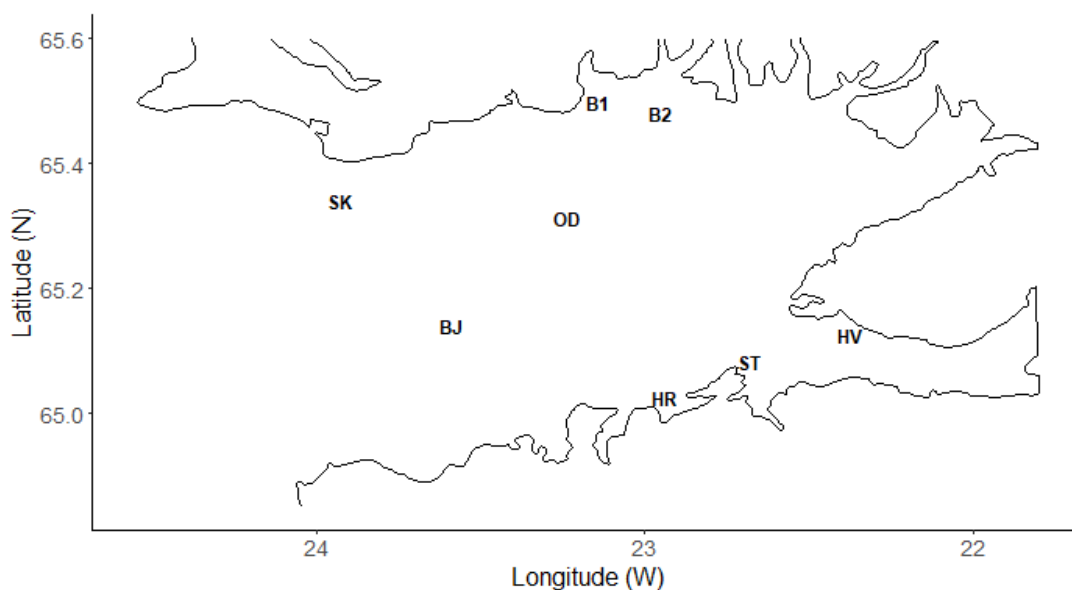
Figure 2-7. A) Setup of the common garden experiment with common whelk juveniles in Hólar University Aquaculture and Fish Biology Lab. B) Juvenile whelk with foot, head and siphon extended. C) Egg-mass. D) Two month old whelk. E) Seven month old whelk. F) 1 year old whelk. G) 2 ½ year old whelk

## 3 Results and discussion

### 3.1 Fine-scale morphological variation of the common whelk – Breiðafjörður, Iceland

Analyses of shell characteristics of the common whelk in Breiðafjörður (Figure 3-1), Iceland, revealed fine-scaled phenotypic differentiation of populations (Figure 3-2), both for adult (**Paper I**) and juvenile whelk raised in the laboratory under standardized settings (**Paper IV**). All variables investigated for adult whelk in the area had a strong correlation with depth; where a gradient of increasing roundness, shell color diversity (Figure 3-3), proportion of striped individuals and thinness of the shell ran from the inner to the outer part of the bay. Shape differentiation between sites increased with geographic distance and depth. Sex, substrate, and proportion of decollated shells were also significantly associated with shell shape.

In the common garden rearing of juvenile whelk (**Paper IV**), excluding the effect of associated environmental variables, differences between Breiðafjörður populations were still significant, emphasizing the role that low population connectivity plays in the population ecology of the common whelk in Breiðafjörður.



*Figure 3-1. Sample sites for adult common whelk in Breiðafjörður, Iceland. Egg-masses were sampled at ST and the surrounding area. Distance between the two closest sites, B1 and B2, is 9 km and the distance between the two furthest sites, HV and SK, is 76 km.*



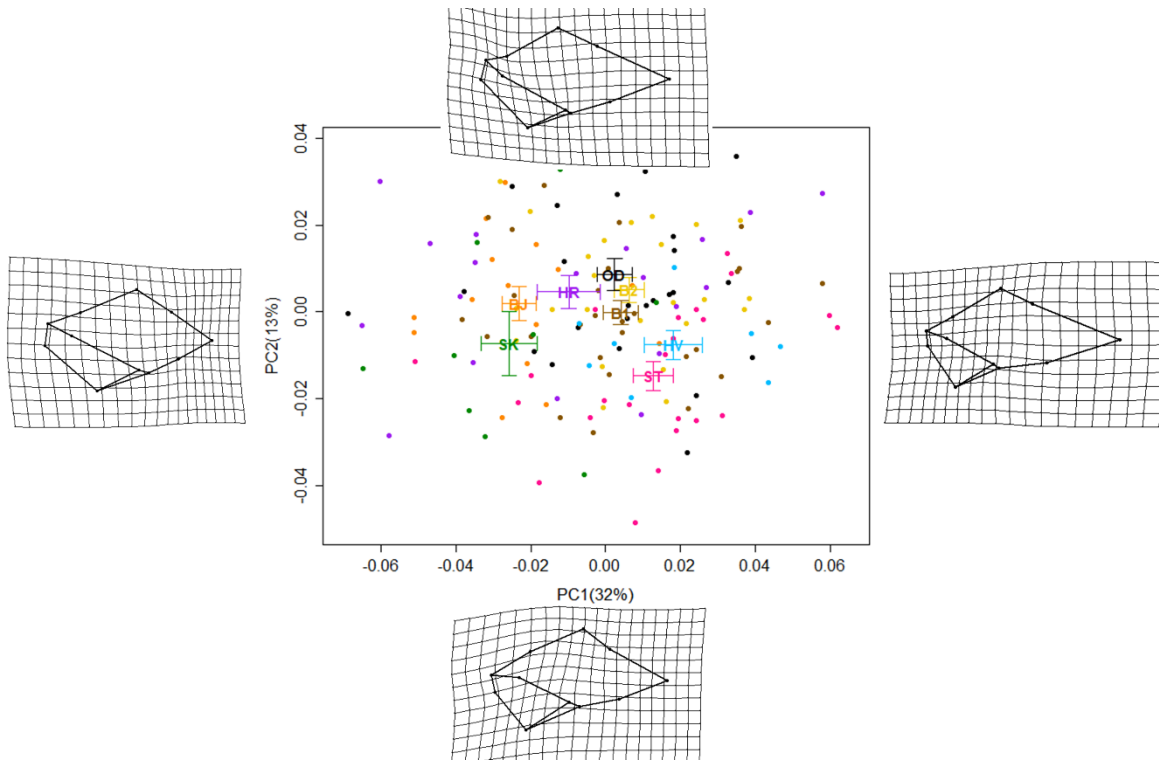


Figure 3-2. Shell shape variation of adult common whelk in Breiðafjörður, Iceland. Percentages given in brackets refer to the proportion of the overall variation explained by the PC-axis. Bars indicate one standard error and site codes are plotted at average PC values of each site. The transformation grids around the graph show the extreme shell shapes along the principal components. Procrustes ANOVA of variation in shell shape revealed a significant effect of both site and sex. Mean PC1 and PC2 values for male and female whelks were (-0.0157, -0.0011) and (0.0079, 0.0005), respectively.

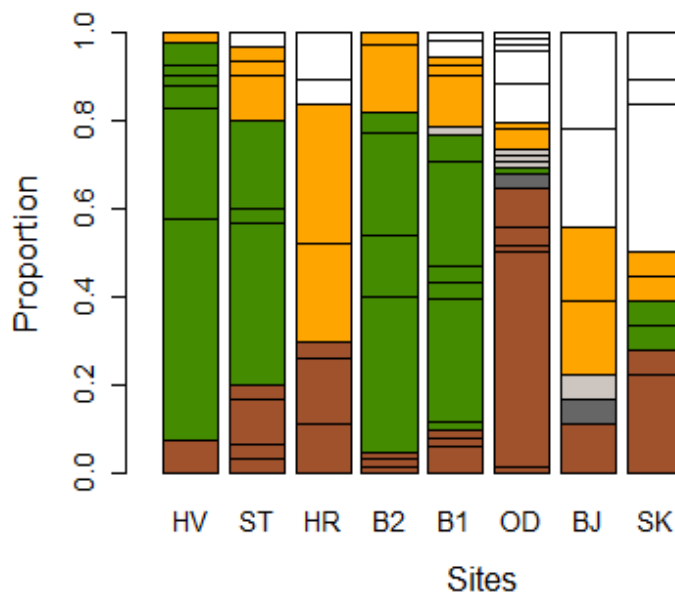
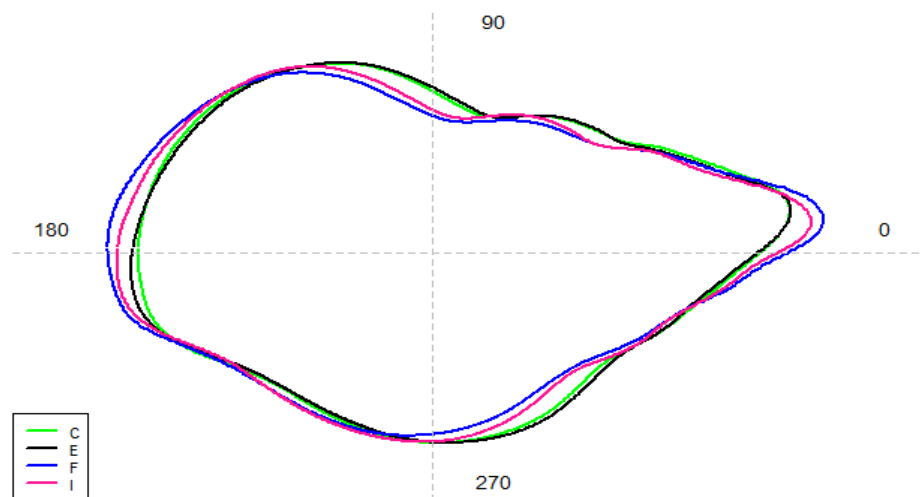


Figure 3-3. Shell color types of the common whelk at sample sites in Breiðafjörður, Iceland, ordered from east to west. Brownish, dark-grey, greenish, grey, orange, and whitish color types are displayed in their respective colors. Horizontal lines within color types show more detailed subtypes within each class of color types.

## 3.2 Large-scale morphological variation of the common whelk – The North Atlantic

Morphological differentiation across the North Atlantic (**Paper III & IV**) reflects the genetic split (**Paper II**) between Canadian, Greenlandic and Eastern North Atlantic common whelk populations (Figures 3-4 - 3-6). Both morphometric methods utilized in the study were mostly in agreement; there was a consistent pattern where differences among populations increased with geographic distance (**Paper III & IV**). Icelandic and Faroese shells for example, had a relatively longer spire compared to the body whorl than Canadian whelk (Figure 3-4) and a narrower body whorl. However, the discordance regarding the English and Canadian whelk between morphometric methods, due to different samples (**Paper III**) emphasizes the importance of taking fine-scaled population structure due to environmental variation into account, particularly since both depth and latitude were correlated with the morphology. Comparison of  $P_{ST}$  vs.  $F_{ST}$  indicated that the observed phenotypic differentiation is most likely the result of genetic drift. Removing some of the environmental variation from the equation by rearing Canadian and Icelandic whelk from the egg-mass stage in a common garden (**Paper IV**) experiment revealed consistent differences between juveniles from the two continents, concordant with the difference observed in wild adults (Figure 3-6).



*Figure 3.4. Mean shell shape (dorsal view) based on Wavelet reconstruction for common whelk from Canada (C), England (E), the Faroe Islands (F) and Iceland (I). Numbers represent angles (in degrees), with the center point indicated by the center of the cross.*

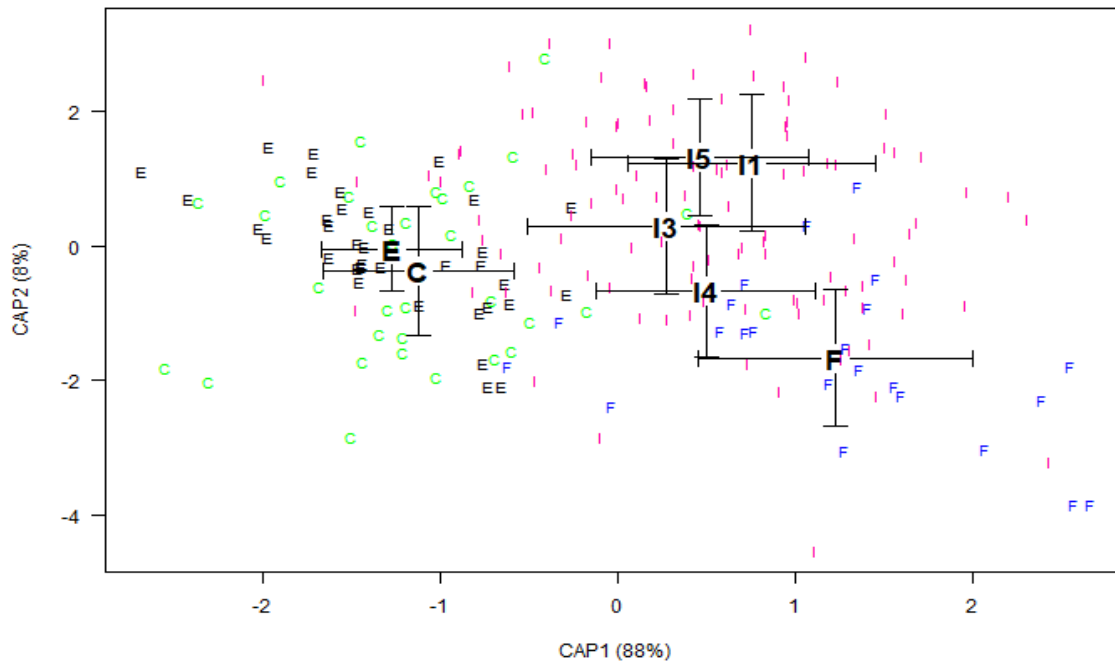


Figure 3-5. Canonical analysis of principal coordinates (CAP1 and CAP2) of Wavelet coefficients of shell shape of common whelk from sampling locations across the North Atlantic. Bold black letters represent the mean canonical value for each population, and colored letters indicate individual whelks. Country of origin is denoted as follows: C, Canada; E, England; F, the Faroe Islands; I, Iceland.

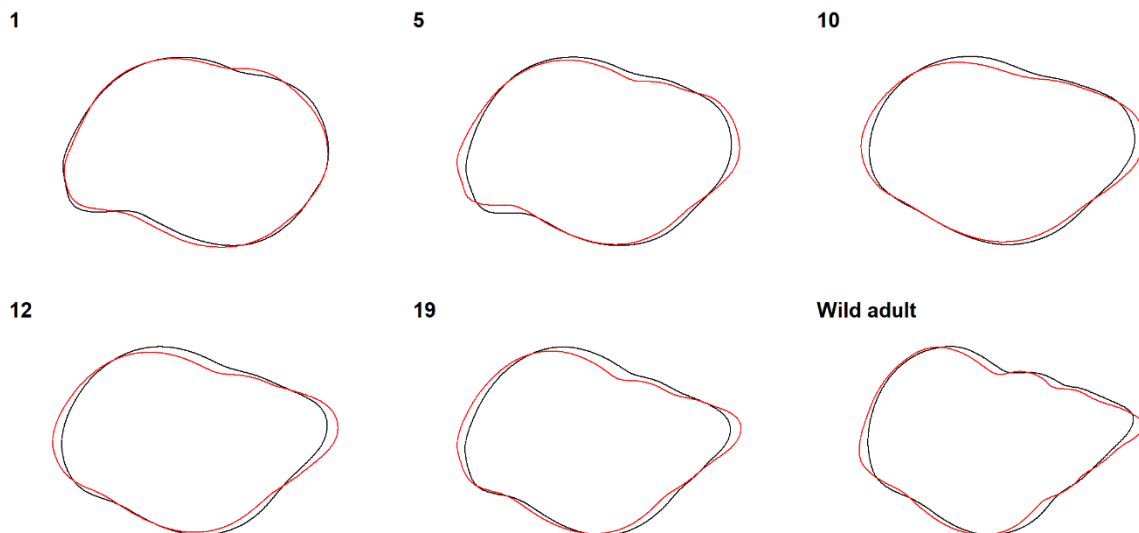


Figure 3-6. Mean shell shape (dorsal view) based on Wavelet reconstruction for common garden reared common whelk from Canada (black outline) and Iceland (red outlines) at the age of 1, 5, 10, 12 and 19 months after hatching, and wild adult whelks (Magnúsdóttir et al. 2019).

### 3.3 Phylogeography of the common whelk in the North Atlantic

Phylogenetic analysis of *COI* variation in North Atlantic whelk (**Paper II**) reflected low or zero connectivity between populations separated by the North Atlantic (Eastern North Atlantic, Greenland and Canada), while over shorter distances genetic differentiation followed an isolation-by-distance model (Table 3-1). The main split between the continents (Figure 3-7) is predicted to have occurred 2.1 Mya (1.04-3.49 Mya), with the Western North Atlantic clade dividing again into two monophyletic clades (Greenland and Canada) 1.3 Mya (0.62-2.14 Mya). The revision of the *COI* data from Pálsson et al. (2014) (*COI-1*) and analysis of the new larger *COI* region (*COI-2*, see **Paper II**) from a more widely sampled area were in agreement on the split between the continents, which was further supported by the species status indices (SSTI and ABGD). The ratio of divergence between and within all three populations exceeded the SSTI that has been proposed for *COI* by Witt et al. (2006), 16.5x compared to 10x, while the ABGD analysis suggested either two (Eastern and Western North Atlantic) or three groups (one in the Eastern and two in the Western North Atlantic, i.e. Canada and Greenland) based on the largest and second largest P-value. Finally, the genetic distances between each of the Canada, Greenland and Eastern North Atlantic populations were similar to or greater than between several other *Buccinum* species (Figure 3-8).

Table 3-1. Pairwise genetic differentiation of *COI-1* mtDNA region in the common whelk across the North Atlantic, both *F<sub>ST</sub>* and  $\Phi_{ST}$  are tested with 1000 permutations.

| Comparison                | $\Phi_{ST}$           |
|---------------------------|-----------------------|
| Canada vs. NE-Atlantic    | 0.991 - 0.995         |
| Canada vs. Greenland      | 0.872                 |
| NE-Atlantic vs. Greenland | 0.916 - 0.943         |
| Within NE-Atlantic        | 0 - 0.42              |
| Comparison                | <i>F<sub>ST</sub></i> |
| Canada vs. NE-Atlantic    | 0.36 - 0.42           |
| Canada vs. Greenland      | 0.36                  |
| NE-Atlantic vs. Greenland | 0.39 - 0.45           |
| Within NE-Atlantic        | 0.008 - 0.159         |

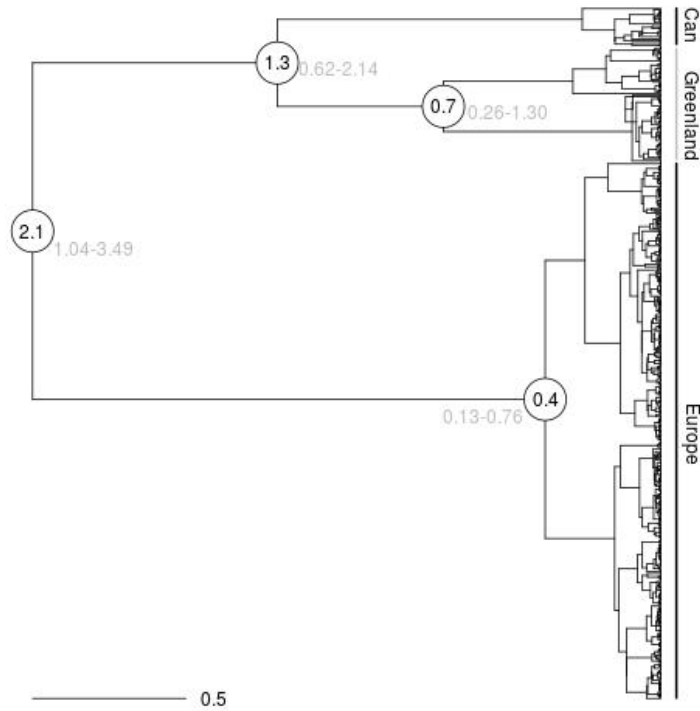


Figure 3-7. Phylogeny of the COI mitochondrial DNA variation in the common whelk across the North Atlantic based on COI-1, a short region of 369 bp (Pálsson et al. 2014). The tree is based on a Bayesian method giving the highest posterior probability using BEAST. The time to the most recent common ancestor of the monophyletic groups (in millions of years) is presented in circles, with the 95% confidence interval adjacent.

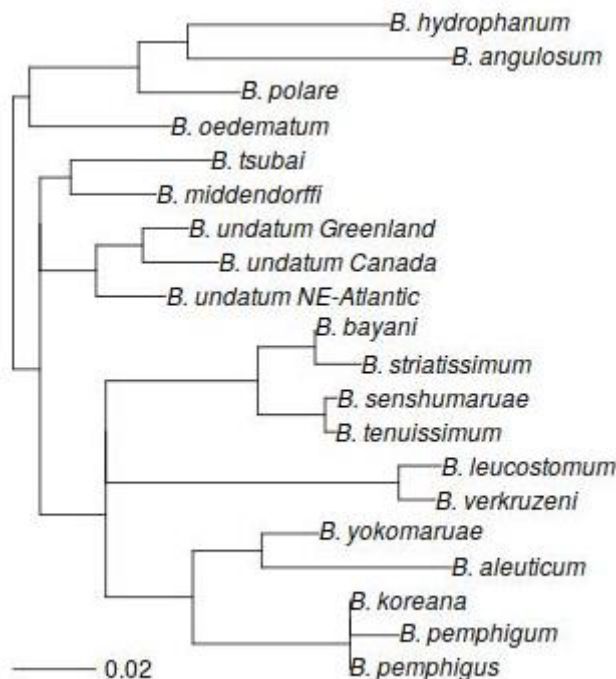


Figure 3-8. Species tree based on COI-1 for common whelk populations in the Western North Atlantic (Canada and Greenland) and Eastern North Atlantic (Europe) and several species within the *Buccinum* genus from the North Atlantic and Pacific Oceans. The tree is based on a 386 bp alignment.

## 4 Conclusion

Drawing on the results presented in this thesis it is evident that a revision of the *Buccinum* genus is necessary, particularly regarding the European and North American common whelk populations. From the revised phylogeography and species status indices of the common whelk across the North Atlantic in **Paper II** together with comparison of juveniles from the two countries in a common garden experiment in **Paper IV** and phenotypic differences between adult whelk from the two continents (**Paper III**), it is evident that the common whelk on either side of the North Atlantic have diverged under allopatry. This is further supported by temporal displacement of mating and development in the two continents (Martel et al. 1986b). Aside from the geographic separation of whelk populations, depth is a common element in all of the current studies, with genetics, shell shape and color all following a depth gradient (**Papers I - III**), which suggests parapatric separation of populations in adjacent areas.

Comprehensive analysis of both phenotype and genotype in adults and juveniles is imperative for consistent species delimitation of shelled marine gastropods. Delimitation of species solely based on shell morphology increases the risk of incorrect estimates of biodiversity and dismisses readily available genetic tools. Furthermore, analyses of phenotypic differentiation should consider fine-scaled phenotypic differences so as not to blur true differentiation, and results from **Papers I and III** support that the methods most sensitive to these differences should be used. Knowledge of true species diversity, particularly with regards to commercially fished species such as the common whelk, is important for proper management and conservation of biodiversity.

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