Age and growth of the Norway lobster (*Nephrops norvegicus*) in Icelandic waters

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90 ECTS thesis submitted in partial fulfilment of a
Magister Scientiarum degree in Biology

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Hér með lýsi ég því yfir að þessi ritgerð er byggð á mínum eigin athugunum, samin af mér og að hún hefur hvorki að hluta né í heild verið lögð fram til hærri prófgráðu.

I hereby declare that this thesis is supported by my research work, written by myself and has not partly or as a whole been published before to higher educational degree.

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Abstract

A lack of extensive age information can lead to a poor understanding of life history and population dynamics. As with most crustaceans, *Nephrops norvegicus* (Linnaeus, 1758) was thought to shed all calcified body parts that could be used to determine age, during periodic moulting. Number of indirect ageing methods have therefore been developed to estimate age. Recently, a direct method to determine the age of crustaceans was developed using growth bands that are deposited in the eyestalk and/or the gastric mill ossicles as age indicator. In this study both indirect length-based methods (ELEFAN I) and new direct method were used to inspect temporal changes in growth of *Nephrops* in Icelandic waters. Two fishing grounds in Iceland were chosen for comparison (Eldey and Breiðamerkurdýpi sites). In recent years the mean size has increased considerably and small animals are almost absent in Icelandic waters. The estimated von Bertalanffy growth parameters from the length-based method varied between time periods and sites (L_∞ = 83–104 mm CL and K = 0.04 – 0.15). Consistent bands were observed with the novel direct age determination method in the zygocardiac ossicle of the gastric mill which were found superior to other ageing structures. *Nephrops* have a long life span and the oldest observed *Nephrops* in this study was estimated to be 22 years old at carapace length (CL) of 86 mm. Site-specific estimated age-at-size curves were developed using growth band counts which suggest a higher growth rate at Eldey site. Comparison between band counts and size-at-age interpretation determined from older length-frequency analysis differ slightly with increased variations for larger animals. The novel direct age determination method has the potential to be immensely valuable for future stock assessment for Icelandic *Nephrops.*

Útdráttur

Upplýsingar um aldur lífvera eru mikilvægar til að skilja lífsögu þeirra og fyrir flest stofnstærðarlíkön. Leturhumarinn, *Nephrops norvegicus* (Linnaeus, 1758), líkt og önnur krabbadýr tapar svo til öllum hörðum vef við hamskipti en það eru þessi vefir sem nýtast til aldursgreininga hjá fiskum og sjávarspendýrum. Því hafa verið þróaðar margar óbeinar aðferðir til að áætla aldur en flestar af þeim byggðar á lengdardreifingum. Nýleg rannsókn sýndi fram á að harðir líkamspartar í augnstilkum og/eða magakvörn (e. *Gastric mill*) krabbadýra tapast ekki við hamskipti og eru þau hluti af líkamsbyggingunni allt til dauðadags. Við nánari athugun hafa fundist rákir í þessum hörðu vefjum sem benda til að þar sé að finna upplýsingar um vaxtarbönd sem eru tengd aldri. Í þessari rannsókn voru vaxtarþættir humars við Ísland áætlaðir með hjálp lengdadreifinga (ELEFAN I) og nýrri aðferð (talin vaxtarbönd) og niðurstöðurnar bornar saman við eldri rannsóknir á vexti ásamt frekari greiningu á lengdardreifingu humars, en undanfarin ár hefur meðallengd humra farið vaxandi og lítið hefur orðið vart við nýliðun. Vaxtaparametrar von Bertalanffy byggðir á lengdardreifingum frá öllum veiðislóðum og tveim samanburðarsvæðum (Eldey og Breiðamerkurdýpi) voru nokkuð breytilegir á milli svæða og tímabila (L_∞ = 83–104 mm SL og K = 0,04–0,15). Nýja aldursgreiningaraðferðin var árangursrík, en skoðaðir voru humrar frá sömu svæðum og áður. Skýr bönd voru sjáanleg í zygocardiac hluta magakvarnarinnar og voru þau mun skýrari en á öðrum svæðum kvarnarinnar. Leturhumar er langlíf tegund og elsti humarinn var metinn 22 ára gamall, en hann var 86 mm á skjaldarlengd. Niðurstöðurnar gáfu til kynna að humrar á Eldeyjarsvæðinu hafi hraðari vöxt en þeir sem eru úr Breiðamerkurdýpi. Eldra aldursmati bar nokkuð vel saman við yngri humra, en hjá stærri dýrum ofmetur eldri aðferðin aldur þeirra. Nýja aldursgreiningaraðferðin býður upp á marga möguleika og gæti orðið mikilvægur liður í stofnmati humars í framtíðinni.

Table of Contents

List of Figures

List of Tables

Acknowledgements

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1 Background

1.1 General biology

1.1.1 Description

The Norway lobster *Nephrops norvegicus*, Linnaeus 1758, is a crustacean decapod of the family Nephropidae, sub-family Nephropinae. It has the usual physiognomy of a clawed lobster and is characterized by its slender body and elongated claws that differ from the "typical" clawed lobsters *Homarus gammarus* and *Homarus americanus* (Figure 1.2) (Bell *et al.*, 2006). Other characteristics include a spiny, ridged cephalothorax, intricately sculptured abdomen and its large, kidney-shaped eyes (hence the genus is named *Nephrops*, which means 'kidney-eye') (Bell *et al.*, 2006; Tshudy, 2013).

Figure 1.1. *Male Norway lobster*.

1.1.2 Distribution

N. norvegicus (henceforth *Nephrops)* inhabit the muddy bottoms of European continental shelves and slopes. It has a wide distribution throughout the northeast Atlantic, ranging from Iceland and Norway in the north of its range, to the Atlantic coast of Morocco in the south including the west and central region of the Mediterranean, at depths from 10 to 800 m (Chapman and Rice, 1971; dos Santos and Peliz, 2005; Farmer, 1975; Figueiredo and Thomas, 1967). Over 30 different populations of *Nephrops* are found in European waters and are fished wherever they are found in exploitable quantities (Ungfors *et al.*, 2013). In Iceland, *Nephrops* are only found in fishable densities off the southern coast on 10 discrete grounds (Figure 1.2) at depths ranging between 100 and 300 m. It is mostly caught at 120-250 m where the bottom temperature is 6-9°C but it varies with seasons (Eiríksson, 1999). The south coast of Iceland is influenced by the warmer Atlantic Gulf Stream making it habitable for *Nephrops* whereas the northern coast is influenced by Arctic waters which keep temperature levels below optimum for *Nephrops*. There are no populations of *Nephrops*

along the northern coast and this division is thought to result from the difference in oceanic conditions (Ungfors *et al.*, 2013).

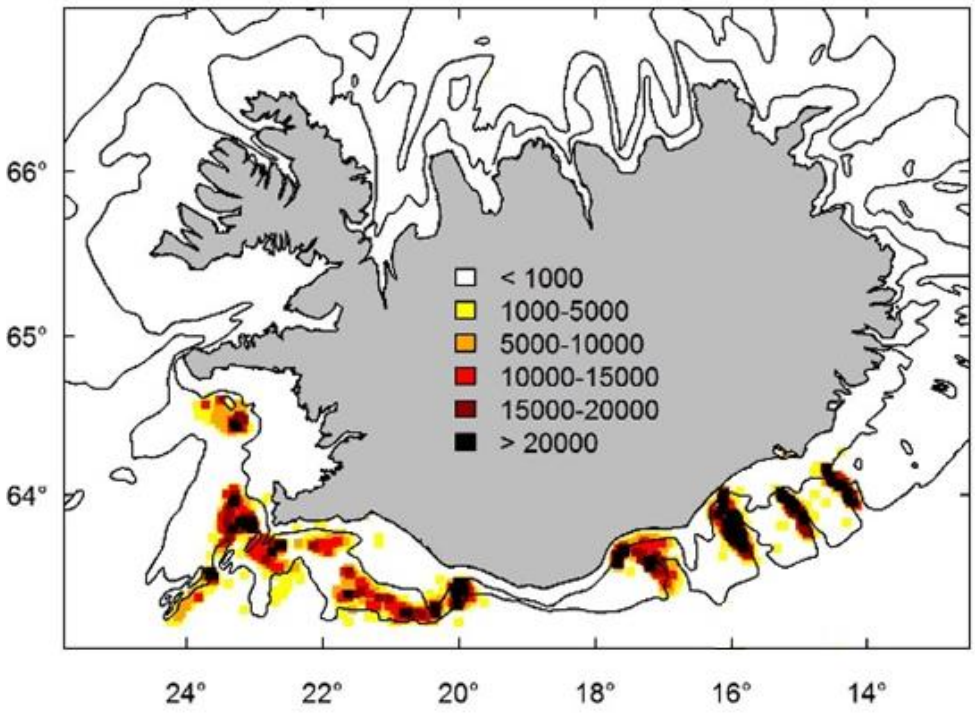

Figure 1.2. Nephrops fishing grounds with catch accumulated from 2000-2015. The colour gradient indicates highest catch (tonnes/nmi2). Contour lines are shown at 100, 200 m.

1.1.3 Ecology of *Nephrops*

Nephrops geographical distribution is highly discontinuous due to their dependency on muddy seabed sediments, with >40% consisting of silt and clay (Bell *et al.*, 2006). On the seafloor, *Nephrops* dig extensive, branching tunnels with two or more crater-like entrance, even though only one serves as the main entrance, and several ventilation shafts (Figure 1.3) (Chapman and Rice, 1971). The burrowing habit of *Nephrops* defines the ecology of the species to a great extent (Chapman, 1980). The lobster's movement about the hole is complex in nature and is determined by several biological factors (sex, size, sexual maturity and time of spawning) and environmental factors (bottom temperature, lighting and clarity). Since most *Nephrops* fishing is done by trawling (although creel fishing is popular in Scotland) and the animal is relatively safe when residing in its burrow, these factors generate massive fluctuation in fisheries. Not only on a yearly-, seasonal- or even daily scale, but in a single day the variation in catch can be substantial (Sarda and Aguzzi, 2012; Thomas and Figueiredo, 1965). *Nephrops* usually exit their burrows only to feed and mate, although larger individuals spend majority of their time foraging (Chapman, 1980).

Figure 1.3. Left: Nephrops burrow with multiple ventilation shafts. Right: Nephrops on a muddy seabed. Images from CoralFish – MRI.

Emergence appears to be related to light and the greatest catches are often taken at dusk and dawn (Aguzzi and Sardà, 2008). Mid-day and at night *Nephrops* generally stay in their burrows. *Nephrops* is an opportunist carnivorous species and a scavenger and feeds mostly on benthic animals and carcasses that fall to the sea floor. Research shows it is a highly unselective feeder and can eat any food particle when it is abundant (Thomas and Davidson, 1962). Outside of its burrow, *Nephrops* are preyed upon by several species. The most influential predator in northern part of its range is cod (*Gadus morhua*) (Chapman, 1980; Farmer, 1975). Other predators include haddock (*Melanogrammus aeglefinus*), hake (*Merluccius merluccius*), whiting (*Merlangius merlangus*), long rough dab (*Hippoglossoides platessoides*), thornback ray (*Raja clavata)* and lesser-spotted dogfish (*Scyliorhinus canicula*) (Bell *et al.*, 2006).

1.1.4 Reproduction

Because *Nephrops* have a wide distribution throughout the north Atlantic the size at sexual maturity and spawning periods varies between regions. *Nephrops* larvae have a long development phase and after fertilization the females (so-called "berried" females) carry their eggs on their abdomen for 6–13 months, depending on latitude and habitat (Farmer, 1974). In most areas however, *Nephrops* spawn annually. In Scotland and Ireland, incubation period is 8–9 months with spawning in the autumn (Farmer, 1974; Figueiredo and Thomas, 1967). Farther south, off the coast of Portugal, the incubation period is even shorter (6–7.5 months) (Figueiredo and Thomas, 1967). Compared with *Nephrops* in lower latitudes, the Icelandic *Nephrops* have a relatively low fecundity and only spawn every other year, with incubation period reaching 12–13 months and therefore the peak of hatching coincides with the peak of spawning in May – June (Eiríksson, 1993). Size at onset of maturity (SOM) seems to be highly habitat dependant. Generally, female *Nephrops* mature at sizes between 23 and 30 mm carapace length (CL, distance from the posterior rim of the eye socket to the

dorsal posterior margin of the carapace) in the shallower waters of north-western and northern Europe, and at sizes 30–36 mm CL in the deep-water population of Portugal (Bell *et al.*, 2006). However, there have been reports on *Nephrops* that fit outside of these estimates. In Irish waters, sexually mature males where observed as small as 15.1 mm CL and females 22.9 mm CL (McQuaid *et al.*, 2006). In a recent study, *Nephrops* caught in Portuguese waters reported the highest value of SOM documented (47–51 mm CL) (Ayza *et al.*, 2011). Sexual maturity in Icelandic waters is reached around 25–30 mm CL (approximately 4 years old) (Eiríksson, 1993). *Nephrops* larvae pass through 3 different larval stages where development is temperature-dependent (Farmer, 1975). After 6 months as larvae (three different larval stages) and post-larvae in the water column, individuals settle on the seabed, where the substrate consists of silt and clay (Aguzzi and Sardà, 2008; Farmer, 1975).

1.1.5 Age and growth

Nephrops growth is characterised by a discontinuous process of moulting and continue to grow after the onset of sexual maturity (Farmer, 1973; González-Gurriarán *et al.*, 1998). Yearly growth, which composes of the rate of moulting and size increase at each moult, can vary greatly with sex and size. In the first year after reaching their benthic stage, both males and females grow quite rapidly and moult approximately once a month (Conan, 1978). After that, intermoult periods get longer and moult frequency decreases to 3–4 times a year until they reach sexual maturity (Bell *et al.*, 2006). The growth slows down considerably after the onset of sexual maturity and moult frequency decreases further to 0–1 moults and 1–2 moults per year for females and males respectively. Females cannot moult without losing their eggs and during the incubation time females are mainly confined to their burrows (Chapman, 1980). Increase in size only happens during ecdyses, with increments at each moult typically range between 1.0 and 2.5 mm CL but smaller and larger values have been seen in many populations (Bell *et al.*, 2006). Males tend to grow much bigger than females and because of the burrowing habits of females, males consist of over 90% of catch landed in Icelandic waters (Eiríksson, 1992, 1993).

1.2 Methods of age determination

Most aquatic animals can be aged by counting annual growth bands deposited in hard structures, such as the otoliths, bones, scales and vertebrae in fishes (Campana and Thorrold, 2001), the genital plates of sea urchins (Agatsuma and Nakata, 2004), shells of bivalves (Kilada *et al.*, 2007; Lutz and Rhoads, 1980) and others (see review Campana and Thorrold, 2001). A major obstacle with determining age of crustaceans is that no similar structure had been found in crustaceans. Given that crustaceans grow by moulting, such a structure was not expected to even exist. In the absence of a direct method for estimating age in

crustaceans, a number of indirect methods have been developed to estimate age (Vogt, 2012).

1.2.1 Rearing in captivity

Determination of the age in captivity is the most exact and reliable ageing technique (Vogt, 2012). However, animals in captivity usually live a rather protected life under optimal conditions. And in addition, growth rate in *Nephrops* is mainly affected by temperature and social interactions (such as density and food availability) (Castro, 1992). Because of this the data collected this way are of limited value to fisheries and ecology. They do however show the upper possible limits of longevity in a species (Vogt, 2012).

1.2.2 Tagging and recapture

Another direct and exact method of age determination is mark and recapture. Once again the problem of moulting can cause external tags to fall off. Methods of tagging so that they were retained at moulting were well developed in 1980 and subsequently, tags that were placed internally followed (Hartnoll, 2001). The major problem with *Nephrops* tag-recapture studies however, is the low recapture rate for tagged animals. Few tag-recapture studies have been reported on *Nephrops* growth and all have recapture rates below 6% (Chapman, 1982; Figueiredo, 1989; Hillis, 1971; Ulmestrand, 2001). A relatively intensive tag-recapture study was conducted in Icelandic waters in 1980 with a low recapture rate of 0.67% (Eiríksson, 1982a). The reason for this low catchability is thought to be due to the burrowing behaviour of *Nephrops* but also the possibility that fishermen do not report an animal with tags (Ulmestrand, 2001). Another problem with the tagging is that there can be no assurance whether the tag has any negative effects on growth or longevity in the species. In addition, tagging experiments are laborious and costly (Bell *et al.*, 2006).

1.2.3 Lipofuscin

The use of lipofuscin provided some promising results for ageing crustaceans (O'Donovan and Tully, 1996; Sheehy *et al.*, 1994, 1999; Wahlel *et al.*, 1996). As a result of routine cellular oxidative processes, lipofuscin is accumulated in neural tissues. Several studies have demonstrated that this accumulation is strongly age-dependent and that deduction of annual cohorts can be achieved using neurolipofuscin concentration-frequency distribution (NCFD) (Fonseca and Sheehy, 2007; Kodama *et al.*, 2006; Sheehy *et al.*, 1999). The lipofuscin method of ageing has repeatedly shown to be superior to size based ageing techniques (Belchier *et al.*, 1998; Sheehy *et al.*, 1994, 1999). This technique however generates context-specific results as the accumulation of lipofuscin is affected directly by individual circumstances such as the environment (Wahle *et al.*, 1996). In a study on the American lobster (*Homarus gammarus)*, it was found that under complex environmental conditions, temperature and chronological age explained at least 93% of individual variation in neurolipofuscin concentration (Sheehy and Bannister, 2002). Therefore the lipofuscin

content is not a marker of chronological age, but rather of physiological age. The average accumulation rate of lipofuscin per year varies among species and some results suggest that if wild population are dispersed, age estimation using lipofuscin could be biased due to differences in diet (Castro *et al.*, 2002; Sheehy *et al.*, 1995). This results in the necessity to construct species-specific databases before application in wild population. Therefore, this method may not become useable in fisheries management (Vogt, 2012).

1.2.4 Length-frequency analysis

Basic information of stock assessment is composed of two major parts; growth parameters that define each stock and the age structure of a population. For crustaceans it has been difficult to estimate these parameters because of the supposed absence of any hard structures that could be used for ageing. The analysis of length-frequency distribution has been the most popular method to determine the age structure of wild crustaceans (Conan, 1978; Farmer, 1973). Length-frequency analysis was developed for species that cannot be aged by a direct approach like growth rings (Jennings *et al.*, 2001). It is built on the identification of modes in the distribution, which can then be related with year classes or recruitment cohorts (Hartnoll, 2001).

Some of the first analytical assessment for *Nephrops* stocks were performed by Jones (1976) using length cohort analysis. The method uses data on the length composition of the catch, together with values for natural mortality and von Bertalanffy growth parameters to estimate stock size and fishing mortality at size. Computer aided methods (such as ELEFAN, MULTIFAN, MIX, etc.) can be of help to identify modes in length-frequency data, but their use often requires interpretation and the resulting outcome may not always be unambiguous (Bell *et al.*, 2006). One of the main benefits with length-frequency data is the collection of data is easy and goes fast. The relatively modest data demands led the method to be widely used to assess *Nephrops* stocks in the northeast Atlantic (ICES, 2001).

Historically many *Nephrops* stocks have been assessed using age-based virtual population analysis (VPA) (Bell *et al.*, 2006), although today UWTV (underwater television) assessments of stocks are thought to be superior (Campbell *et al.*, 2008; Sarda and Aguzzi, 2012). VPA uses fishery-dependent data to determine the past stock size using mortality rates (Jennings et al., 2001). VPA also needs age-based data for population dynamic modelling to describe the influence of fishing pressure upon the stock size. Without reliable age-data, the VPA uses the von Bertalanffy growth parameters to convert length data into age classes (Bell *et al.*, 2006; Ungfors *et al.*, 2013). In applying the VPA technique to numbers caught in each varying-sized length group that is estimated to compose a certain virtual "age group", a number of input values are required such as L_∞ (asymptotic length), M (natural mortality) and K (growth coefficient). The cohorts "year-classes" are formed by slicing the length-frequency distribution into knife-edged demarcations (Eiríksson, 1979). This method has been criticised because of the lack of assumed variability in growth, and

other external influences such as sampling and capture methodology (Ulmestrand, 2001; Sarda and Aguzzi, 2012). Nevertheless, when combined with other sources of information on stock trends, such as catch and landings per unit effort (CPUE and LPUE) trends, VPA has proved successful (ICES, 2003, 2004).

Although these approaches have proven useful, they have some limitations (Vogt, 2012). Because of environmental effects on growth rate, for example changes in temperature (Tamm & Cobb, 1978), the size-age relations these methods establish can only be applied to the years and population under examination. In addition, size frequency data give reliable information for short-lived species with well-defined annual reproduction periods. The longer a species lives the method becomes increasingly unreliable because slowly growing specimens of older age may group together with fast growing specimens of younger age. After the onset of sexual maturity, when growth rate drops down and modes of successive age classes start to contort, the identification of age cohorts become difficult (Bell *et al.*, 2006). Since these effects become bigger with the extension of life span, size-frequency based growth models are unsuitable for very long-lived species (Hartnoll, 2001; Sheehy *et al.*, 1999). Despite all its disadvantages, size-frequency related growth models are the most convenient and cheapest approach to analyse the age structure of populations that lack age-information bearing structures. “Therefore it will remain the prime source of information on longevity in decapod crustaceans, at least until something better comes along” (Vogt, 2012).

1.2.5 Aims

The aims of this study is to analyse the age and growth of *Nephrops* in Icelandic waters. It is broken up into two chapters. In chapter one we investigate the usability of length frequency analysis to estimate growth in a long lived species such as *Nephrops* and explore what length frequencies can tell us about changes in the population. In chapter two we utilize a new direct ageing method, estimate the repeatability of such a method and how practical it is for regular monitoring of *Nephrops* stocks. In addition we will corroborate the results from the new ageing method with previous age-length estimates to investigate the credibility of previously estimated age based on length data. Two important *Nephrops* fisheries sites were chosen for sampling to further explore whether environmental changes affect *Nephrops* that grow at different temperature levels.

2 Decadal developments of length frequencies and growth parameter estimation from *Nephrops norvegicus* in Icelandic waters

2.1 Introduction

Age information along with growth, and mortality rate are viewed as the most important biological characteristics controlling the productiveness of stock populations (Campana and Thorrold, 2001). Because crustaceans grow by moulting, growth and population studies have always been restricted by the absence of direct age determination methods. The importance of growth information has generated many alternative techniques to direct age estimation such as; tag-and-recapture methods (Chapman, 1982; Eiríksson, 1982a; Figueiredo, 1989; Ulmestrand, 2001), lipofuscin accumulation (Sheehy, 1989; Wahlel *et al.*, 1996; Maxwell *et al.*, 2007) and size-frequency analysis (Castro, 1995; Farmer, 1973; Figueiredo, 1984; Hillis, 1971) (see greater details in chapter 1.2). Of these, the most commonly used in *Nephrops* research is length-frequency analysis. As a consequence, methods to assess Icelandic *Nephrops* stock size is largely based on length-frequency data. Early assessments of Icelandic *Nephrops* stocks were based on surplus production models which relied on catch per unit effort and landings data and gave an indication of the maximum sustainable yield (MSY) (Eiríksson, 1974). Jones (1976) was the first to describe the preliminary "steady state" assessment of the Firth of Forth *Nephrops* stock in East Scotland, using only length composition data and no precise data on age (Jones, 1976). As a result, experiments on annual cohort analysis and estimations on the von Bertalanffy growth parameters were performed in Icelandic waters (Eiríksson, 1982b). Such slicing of length distributions of total *Nephrops* catches have been carried out annually since 1977 (Anon, 2015; Eiríksson, 1982b, 1992).

In order to utilize length–frequency analysis of any kind, ample amount of samples are required. The Icelandic trawl fishery for *Nephrops* first started in the late 1950s and CPUE (landings per hour of trawling) has been monitored via fishery log-books since 1960. In addition, sampling of catches (length compositions by sex) date back to 1959 (Sigurðsson, 1965). Besides sampling of logbook data, the MRI (Marine Research Institute of Iceland) conducts an annual *Nephrops* survey on all known *Nephrops* grounds (Figure 1.2). Scientific surveys have the benefit over commercial catch statistics in that they are undertaken in controlled environment and fully directed at a target species (Bell *et al.*, 2006). However, limits on time and resources mean that the many factors, such as weather conditions and sea state, have an effect on catch rates (Maynou and Sardà, 2001). Considering the strong diurnal

patterns in catch rates of *Nephrops* (Chapman, 1980; Sarda and Aguzzi, 2012) and the reasons above, trawl surveys are often considered to be an unsatisfactory method of measuring stock trends. (Bailey *et al.*, 1993; Bell *et al.*, 2006). In recent years, underwater television (UWTV) surveys have become more frequent, but they are thought to produce the most reliable stock assessments by counting *Nephrops* burrows and it has become a standard for assessing *Nephrops* populations in UK and Irish waters (Campbell *et al.*, 2008; Sarda and Aguzzi, 2012). No such survey has been conducted in Icelandic waters to date. Underwater television estimates of burrow density have however been strongly correlated with catch rates in trawls which have coincided with a peak burrow emergence (Smith *et al.*, 1997). Trawl catch rates can therefore provide a useful index of local abundance (Tuck *et al.*, 1997).

The use of length-frequency information to study growth depends on the identification of modes in the distribution, at least at small sizes, which can be equated with year cohorts or recruitment events (Hartnoll, 2001). The length of these modes are taken to be the mean lengths for the assigned cohorts, and these mean length can then be used to fit growth curves. The most accurate information about growth patterns can be obtained by having length-frequency data over long time periods. With a long time series, samples can be correlated and corrected for unordinary modes created by sampling problems and strong modes can be followed through time to provide direct reconstruction of historical growth patterns (Hilborn and Walters, 1992). Various methods have been developed for analysing growth patterns from length-frequency samples (Hartnoll, 2001; Vogt, 2012). Among these, the ELEFAN I routine implemented in the FiSAT software has been most frequently used for estimating population parameters of species that are difficult to estimate age directly, especially in tropical and subtropical fish species and also crustaceans, primarily because it requires only length-frequency data (Pauly and David, 1981).

Most growth studies on *Nephrops* have obtained growth rates of around 0.1–0.2 years^{-1} (Eiríksson, 1982a; Mytilineou and Sarda, 1995; Sardà, 1998; Tuck *et al.*, 1997). Furthermore, it has been reported that biological parameters such as growth, population density and size–frequency distribution may differ between and within populations (Bailey and Chapman, 1983; Tuck *et al.*, 1997). These variations could be associated with changes in oceanic conditions, sediment type, food availability (Bell *et al.*, 2006; Chapman and Bailey, 1987; Tuck, 1993) or fishing pressure (Sánches Lizaso *et al.*, 2000). Over the past decade, elevation in ocean temperature has been detected in Icelandic waters and at the same time the amount of *Nephrops* larger than 70 mm is increasing and low recruitment numbers in *Nephrops* population have been reported (Anon, 2015; Valdimarsson *et al.*, 2012).

In this study, the objective was to inspect recent changes in *Nephrops* populations using length-frequency analysis. Time series of length-frequencies were analysed and historical changes in length estimated. Growth parameters were estimated for different time periods and sites and newer growth parameters compared with estimates from older growth studies.

2.2 Material and methods

2.2.1 Data collection

Data in this study was obtained from the annual *Nephrops* surveys conducted by the MRI in the years 1987 – 2015. The research areas cover all *Nephrops* fishing grounds from the westernmost area Jökuldjúp to Lóndsjúp in the East (Figure 2.1). Around 55 tows were conducted every year in May. Each tow was approximately 2 hours or 5 nm. The gear was standard *Nephrops* trawl with 80 mm mesh size in the cod end.

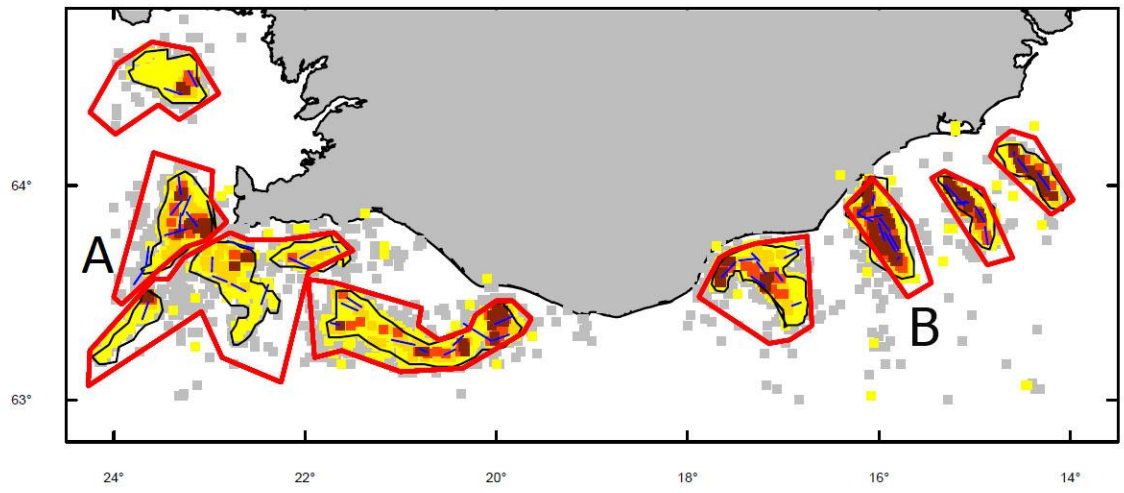

Figure 2.1. Nephrops survey areas. Red lines outline designated Nephrops sites. Eldey-site (A) and Breiðamerkurdýpi-site (B) are indicated with black letters. Survey tows are marked with blue lines. The colour gradient (yellow-dark red) is derived from logbook data, where grey/yellow = single/low catch and red = high catches.

Individuals were sexed and their body size measured (to the nearest 1 mm) as carapace length (CL) using vernier calipers. Males consist of more than 90% of catch landed in Icelandic *Nephrops* fisheries and were therefore preferred in this study (Eiríksson, 1979). Two of the largest *Nephrops* fishing grounds in Iceland (Eldey (9 tows) and Breiðamerkurdýpi (9 tows) sites) were chosen for comparison along with all other sites combined and used in length-frequency analysis. All length-frequency analysis were performed using the computing environment R (R Development Core Team, 2015).

2.2.2 Estimation of growth parameters

Growth parameters were estimated using FiSAT II software (Gayanilo *et al.*, 2005) and the ELEFAN I package (Pauly and David, 1981). Data from *Nephrops* surveys (1987 – 2015) was split up into 3 different periods (1987 – 2000, 2001 – 2015 and 1987 – 2015) (table 2.1)

to explore temporal patterns in growth parameters. ELEFAN I has a maximum capacity of 50 consecutive lines in a data set so length groups from 24–73 mm CL were selected to represent the *Nephrops* population.

In ELEFAN I, data is reconstructed to generate highest limits ("peaks") and lowest limits ("troughs") and the goodness of fit index (R_n) is defined by:

$R_n = 10^{ESP/ASP}/10$

where the ASP ("Available Sum of Peaks") is computed by adding the 'best' values of the available 'peaks' and the ESP ("Explained Sum of Peaks") is computed by summing all the peaks and troughs "hit" by a growth curve of the form,

$$L_t = L_\infty\big(1 - EXP(-K(t - t_0) + S_{ts} + S_{t0})\big)$$

where

$$S_{ts} = (CK/2\pi) \times \sin\big(2\pi(t - ts)\big)$$

$$S_{t0} = (CK/2\pi) \times \sin\big(2\pi(t_0 - ts)\big)$$

where L(t) is the length at time t (years), L_∞ the asymptotic length and K is referred to as body growth coefficient. The parameter t_s is the summer point and the parameter C is the magnitude of seasonal variation (0–1). If C=0, the equation reduces to the ordinary (non-seasonal) von Bertalanffy equation, where C=0 implies that there is no seasonality in the growth rate. In this study no seasonal data is available and therefore the non-seasonal von Bertalanffy equation is utilized. The ELEFAN I method generates an 11 by 11 matrix showing R_n values and in which the 10 best values are highlighted, to better enable selection of the "best" combination of growth parameters. The highest value of R_n and any value within a 2-3% range of it were chosen to select the parameters that best described the data. Since the parameters L_∞ and K are inversely correlated, variation in growth performance was analysed by using the phi prime index (Munro and Pauly 1983) where:

$\phi = \log_{10}K + 2\log_{10} L_\infty$ mm

For comparison the phi-prime index was also calculated for previous growth studies in Icelandic waters (Eiríksson, 1982a).

2.3 Results

2.3.1 Length frequency distribution

Length frequency distributions of *Nephrops* did not vary extensively during the period of study (1990 – 2010) in all sites combined (Figure 2.2). Overall, the mean sizes ranged from lowest point of 40 mm CL to approximately 50 mm CL. Strong pulses of recruitment or small individuals were observed in the years around 1995 and 2002. During the former period there was a shift to smaller mean sizes, in the latter period there was also an increase in larger size classes resulting in a more stable mean size. In 2010 the mean size increased every year and in 2015 it was at its highest value over the whole time series (56 mm CL). Also during this period, there was a rise in the 2% quantile and in 2015 it exceeded 35 mm CL for the first time.

At the Eldey site the mean sizes had fluctuated more extensively than the overall average (Figure 2.3). There was an abrupt peak above 60 mm CL in 2003, when few but large *Nephrops* were caught in the survey. The mean size declined again until 2010, when it started to rise and was approximately 51 mm CL in 2015. The 2 % quantile does not increase in the same way as the mean size and remains stable. The 98 % quantile was generally high in Eldey site and surpassed the 60 mm in 1993 with no indication of significant declining.

In Breiðamerkurdýpi site the mean size showed periodic fluctuations between 38 and 45 mm CL from 1990 – 2010 (figure 2.4). In 2010 the mean size increases and was approximately 52 mm CL in 2015. The 98 % quantile was generally low for this area and does not exceed the 63 mm until in 2010. The 2 % quantile followed the same trend as mean size and ranged between 28 and 33 mm CL until it increases drastically in 2010. The size frequencies show occasional well defined modes, for example in 1989 – 1992, 1996 – 2000 and again in 2002 – 2005, which may be linked to strong year classes (Figure 2.2 and 2.4).

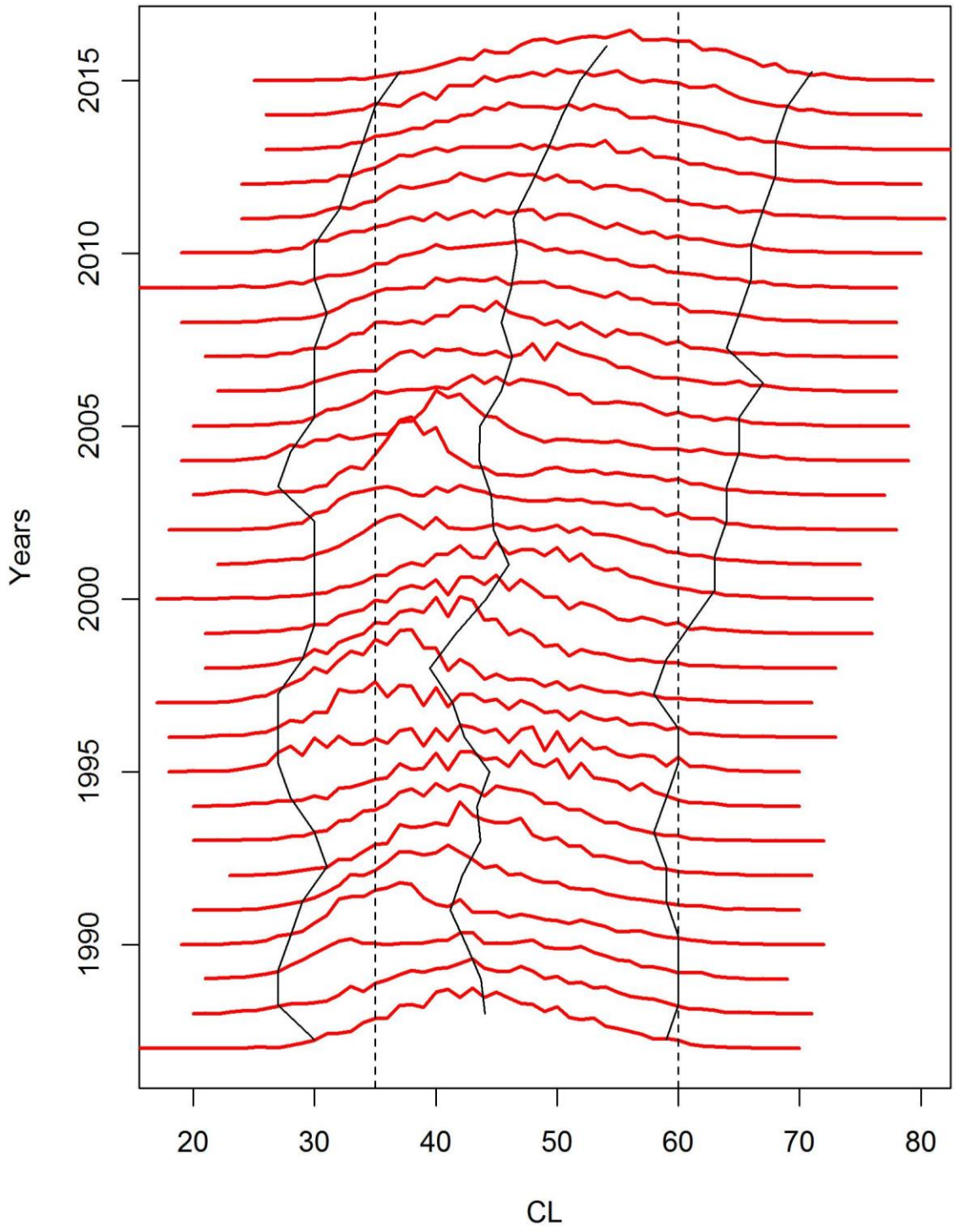

Figure 2.2. Time series of length-frequency distributions for Nephrops in all assessment areas between 1987 and 2015 from MRI survey data. Black lines represent the average size and the 2 and 98 % quantiles for each year. The dotted vertical lines are fixed minimum targeting size (35 mm CL) and what is considered as large (60 mm CL (+14 years old)).

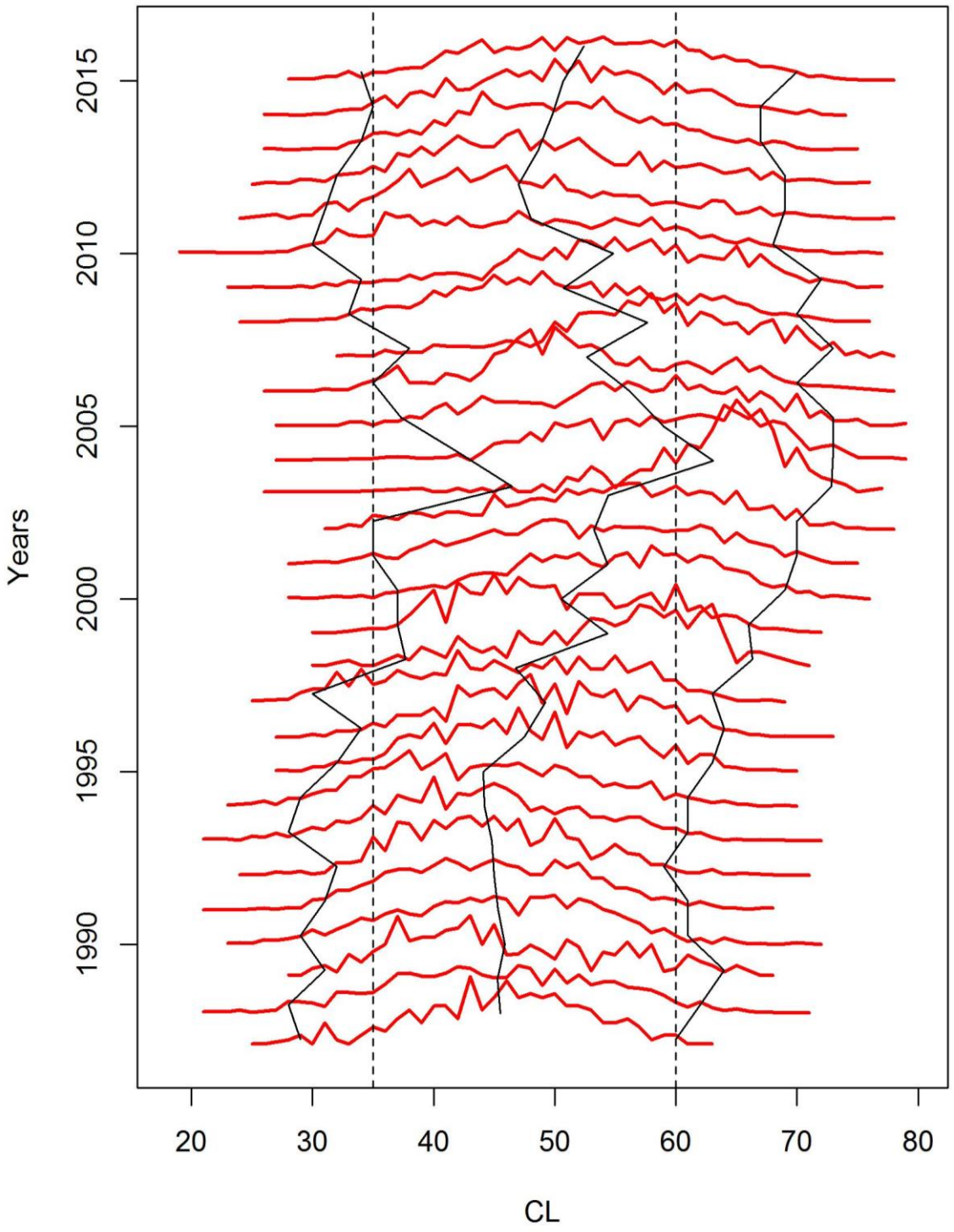

Figure 2.3. Time series of length-frequency for Nephrops in Eldey assessment area between 1987 and 2015 from MRI survey data. Black lines represent the average size and the 2 and 98 % quantiles for each year. The dotted vertical lines are fixed minimum targeting size (35 mm CL) and what is considered as large (60 mm CL (+14 years old)).

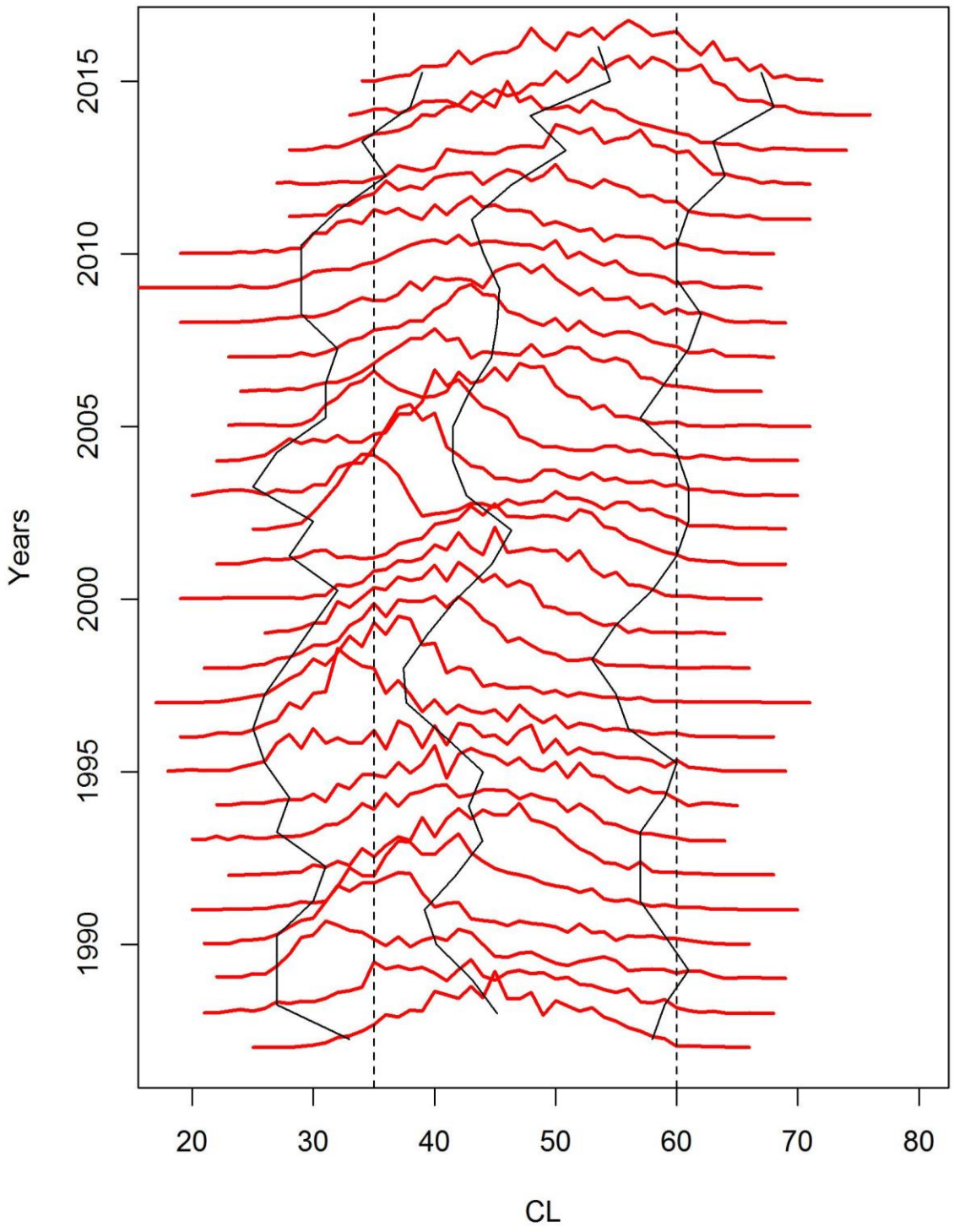

Figure 2.4. Time series of length-frequency for Nephrops in Breiðamerkurdýpi assessment area between 1987 and 2015 from MRI survey data. Black lines represent the average size and the 2 and 98 % quantiles for each year. The dotted vertical lines are fixed minimum targeting size (35 mm CL) and what is considered as large (60 mm CL (+14 years old)).

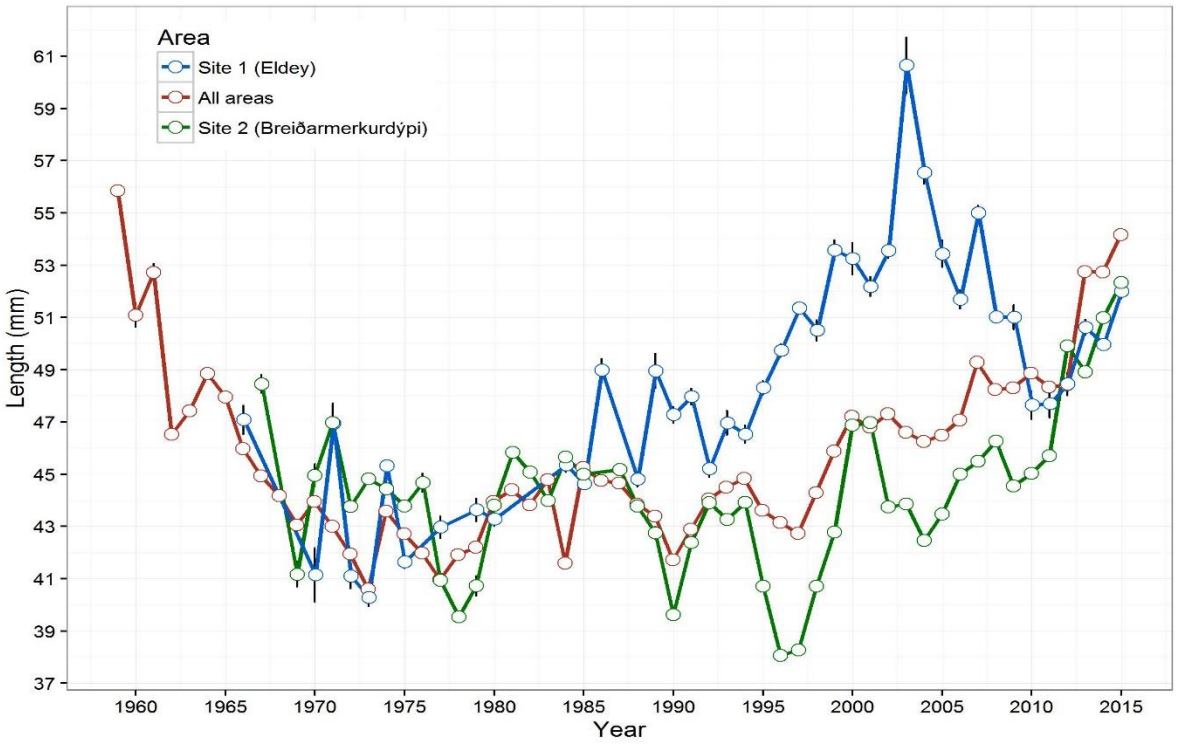

Figure 2.5. Historical mean size (CL) distributions of Nephrops since the beginning of fisheries recordings in 1959.

After the onset of *Nephrops* fisheries the mean size declined from roughly 55 mm CL in 1959 to around 44 mm CL in 1970 for all areas combined. Relatively long period of stability was observed between 1970 and 1995, but in recent years the mean size has been more variable (Figure 2.5). In 1996 an increase in mean size is seen at all sites from 45 mm CL to 53 mm CL in 2015. Eldey site shows greater fluctuation with mean size rising to 60 mm CL in a short period (2001 – 2003) and declining again in 2004. Breiðamerkurdýpi site reaches a low point in 1990 (40 mm CL) and in 1996 (39 mm CL) before rising to the same mean size as Eldey in 2015 (51 mm CL). Mean sizes of all *Nephrops* sites combined are now the similar as when fisheries began.

2.4 Growth estimates (ELEFAN I)

The estimates of the von Bertalanffy parameters from the ELEFAN I method varied between periods and sites (Table 2.1). The L_∞ values ranged between 83 and 104 mm CL and the growth parameter *K* was estimated to be between 0.04 and 0.15.

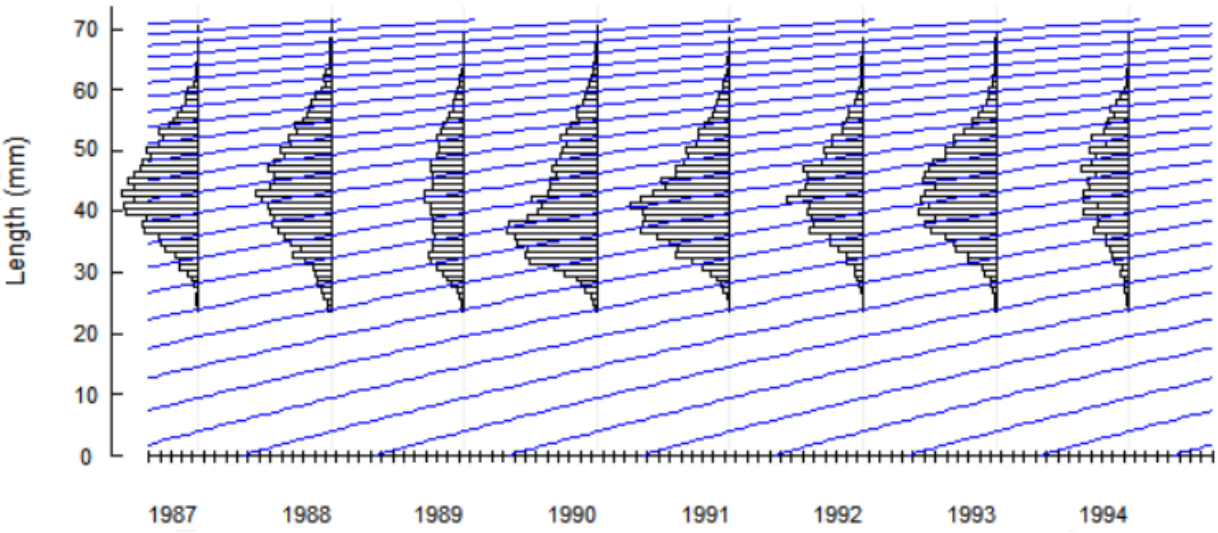

Figure 2.6. Nephrops yearly length-frequency distributions for the years 1987-1994 in all areas. The blue von Bertalanffy growth isopleths are based on the following parameters: L_∞ *= 98 mm CL, K= 0.06 and* R_n *= 0.206*

Table 2.1. Growth parameter estimates (L_∞ and K) and the goodness of fit index (R_n) for nine different time-site combinations generated using ELEFAN I method. ϕ = growth performance index.

	Area	L_∞	*K*	R_n	ϕ’
1987-2000	*Eldey*	89	0.11	0.156	2.94
	Breiðamerkurdýpi	88 – 92	0.11	0.176	2.95
	All sites	98 – 101	0.06	0.206	2.78
2001-2015	*Eldey*	83 – 84	0.13	0.155	2.96
	Breiðamerkurdýpi	104	0.06 and 0.15	0.135 and 0.137	2.81 and 3.21
	All sites	101	0.04	0.162	2.61
1987-2015	*Eldey*	83	0.12	0.143	2.92
	Breiðamerkurdýpi	88 – 97	0.06	0.147	2.71
	All sites	86 – 95	0.05	0.173	2.63

Estimates of *K* were stable between 0.11–0.13 at Eldey site and L_∞ was estimated to be between 83 – 89 mm CL. At Breiðamerkurdýpi site there was more fluctuation in the best estimates. In the latter period (2001 – 2015) two of the best values of *K* were far apart, or 0.06 and 0.15 respectively, with rather high, 104 mm CL, value of L_∞. For all sites combined the best estimation for *K* was generally lower than in the two sampling sites (0.04–0.06) and L_∞ between 86–101 mm CL (Table 2.1). The highest goodness of fit index (R_n = 0.206) was obtained when K was 0.06 and L_∞ was estimated 98–101 mm CL in the earlier period (1987 – 2000) for all sites.

Values of the growth performance index, ϕ’, were between 2.6–2.9 for all areas and indicate no unusual variation in growth rate, except at Breiðamerkurdýpi site during latter period (ϕ’= 3.2 when *K* = 0.15).

2.5 Discussion

In the last five years, apparent changes in length-frequency distribution of *Nephrops* population in Iceland are evident as mean size has increased and small *Nephrops* are missing from the population samples. Mean sizes in catch samples can be informative about changes in population processes. Increase in mean size could reflect either declines in recruitment, i.e. diminishing numbers of small individuals in the population, or decreased mortality, i.e. increased numbers surviving to attain the larger size classes, and the opposite explanation could apply to decreases in mean size (Bell *et al.*, 2006). In 1990 and 1996 two sharp decreases can be seen in mean sizes of all sites combined and Breiðamerkurdýpi. In the same time period, strong cohorts can be seen in the length-frequency time series. These low points are most likely due to strong recruitment years.

In 2011 the inter-site differences decline and at same time the mean sizes increase. In 2015 recruitment appears to be low at all sites combined. This includes Breiðamerkurdýpi and is a likely cause for increases in mean size. The only areas where some recruitment has been detected in recent years is in Eldey site and Skerjadjúpi (SW corner) (Anon, 2015). Eldey site has always produced large Nephrops with 98% quantile above 60 mm CL since 1993. A spike in mean size was observed in 2003. This was most likely due to low sample size in that time period. When sample sizes are low in *Nephrops* surveys it is common to see larger individuals dominate in the small catches (Jónas Páll Jónasson, pers. comm.). The reason for the unusually high mean lengths in Eldey site are also possibly due to very low fishing pressure over a nine year period (1998 – 2006) (Anon, 2015). The low fishing pressure is due to low CPUE in that area but also because of good conditions and high fishing pressure at the eastern sites (Anon, 2015). Sánchez et al. (2000) suggested that growth rate may also increase when fishing pressure decreases due to the negative effect overfishing has on the population density. There is a change in growth rate in the Eldey site between the two time periods 1987 – 2000 and 2001 – 2015, were K= 0.11 and K= 0.13, respectively. In recent years, sea surface temperature in Icelandic waters has increased by 1 to 2°C in the waters south and west of Iceland (Valdimarsson *et al.*, 2012). These changes could have an effect as well because temperature is considered to be one of the main influencing factor on growth of crustaceans (Hartnoll, 2001). The effect of increased water temperature on growth can be seen in reduced time to reach certain stages of the life cycle (onset of sexual maturity or the end of larval development), also these stages are attained at a reduced size (Hartnoll, 2001). The growth rate in Breiðamerkurdýpi site also differs between time periods, K = 0.11 and K= 0.06/0.15. These differences in addition to a high L_∞ (104 mm CL) suggest that the parameters in the second period are questionable. Furthermore, in the latter time period, low R_n values might indicate that the parameters are not accurate.

The R_n values for all sites combined are relatively high in all time periods which suggest that the parameters describe the data reasonably well. L_∞ is estimated from 86–101 mm CL and the estimation of K is between 0.04 and 0.06 which is low. Since a negative relation exists between L_∞ and K, the low values of K could also be related to the unusually high L_∞ values that were observed. Data from older *Nephrops* studies in Icelandic waters estimated the von Bertalanffy growth parameters as K= 0.101 and L_∞= 80 mm CL (Eiríksson, 1982a). Values of the growth performance index (ϕ') that was calculated for all estimated parameters in this study and for older parameters are however similar 2.6–2.9 and 2.81, respectively. Reasons for the variation in parameters are hard to explain and could be related to changing environment in Icelandic waters (increase in temperature for example). It is however consistent with other studies on *Nephrops* that show that the growth rate can vary considerably due to environmental factors such as temperature, sediment particle size, food availability, population density and fishing pressure, each of which may have different and possibly interactive effects (Bell *et al.*, 2006; Tuck, 1993). Seabed sediments with high silt and clay content, and therefore high food supply, tend to support the low-density populations of fast growing animals (Chapman and Bailey, 1987). However, a high population density implies large intraspecific competition for food, which may limit growth (Parslow-Williams *et al.*, 2001). With these complex factors interacting as they presumably do, it is difficult to estimate which factors are of importance in Icelandic waters.

The samples used in this study were obtained from the annual *Nephrops* trawl survey where the purpose is to estimate the stock size of fishable *Nephrops.* Therefore the same mesh size of 80 mm is used as in commercial fisheries and most individuals smaller than 35 mm CL were excluded from the trawl. Hilborn and Walters (1992) point out that when using the ELEFAN I method the generated size distribution is a product of gear selectivity and growth. Furthermore, the fishing gear has to be totally unselective to produce an accurate growth curve (Hilborn and Walters, 1992). The ELEFAN I method was originally intended for short-lived animals with well-defined size distribution modes. Mathews (1987) suggested that approaches based solely on length data become increasingly more unreliable as the species become older. Furthermore, growth parameters estimated by ELEFAN I become increasingly biased when the overlap between adjacent length-frequency distributions increases (Mathews, 1987). This bias is expressed in overestimation on L_∞ and the underestimation of K, with the growth curve being very similar to the correct curve however (Hampton and Majkowski, 1987; Rosenberg and Beddington, 1987). The best solution to this problem is either the use of additional information on growth (such as derived from tagging data) or, preferably, age-length data obtained by reading otoliths (Pauly, 1987). Which brings us back to the original problem; "while size measurements can be useful for some purposes other than age determination, such as indices of fecundity or condition, knowing an individual's age is what really counts." (Fonseca and Sheehy, 2007).

Recently, a new direct ageing technique to determine the age of crustaceans was developed where cuticle growth bands were observed in the eyestalk and/or the gastric mill ossicles of

four crustacean species, including the American lobster (*Homarus americanus*) (Kilada *et al.*, 2012). This method has also been applied on several other crustacean species (Kilada and Acuña, 2015; Kilada and Ibrahim, 2016; Leland *et al.*, 2011, 2015) including *Nephrops* (Kilada *et al.*, 2015) with promising results. This method has a potential to be valuable in various fields, especially in fisheries management and notably for long lived species where length based methods have proven to be difficult.

3 Estimated age of the Norway lobster (*Nephrops norvegicus*) in two sites in Iceland using gastric ossicular growth-band counts

3.1 Introduction

For management of commercially valuable species, accurate age estimates are among the most important assets (Figueiredo, 1984). Most crustaceans were thought to lose any calcified markings that could be used to determine age, during periodic moulting. As a consequence, many methods have been applied to acquire age estimates for crustaceans i.e. size-frequency (Castro, 1995; Farmer, 1973; Figueiredo, 1984; Hillis, 1971), tag-and-recapture (Chapman, 1982; Eiríksson, 1982a; Figueiredo, 1989; Ulmestrand, 2001), lipofuscin accumulation (Sheehy, 1989; Wahlel *et al.*, 1996; Maxwell *et al.*, 2007) and other biochemical approaches such as radiometric ageing (Verdoit *et al.*, 1999). None of these methods provide a direct age assessment of individual crustaceans. Compared with direct ageing methods, like those using fish otoliths and scales, mollusc shells (Kilada *et al.*, 2007) and other calcified body parts (for review, see Campana and Thorrold, 2001), they are inferior. Recently, a direct method to determine the age of crustaceans was developed using growth bands that are deposited on the eyestalk and/or the gastric mill ossicles as an age indicator (Kilada et al., 2012). The authors produced, for the first time, the sex-specific size-at-age relationship in four species including the American lobster (*Homarus americanus*). The same method was also applied on several other crustaceans (Kilada and Acuña, 2015; Kilada and Ibrahim, 2016; Kilada *et al.*, 2015; Leland *et al.*, 2011, 2015) and has a potential to be immensely valuable to various crustacean commercial fisheries.

The Norway lobster, *Nephrops norvegicus*, Linnaeus 1758, has a wide distribution throughout the northeast Atlantic, ranging from Iceland and Norway in the north, to the Atlantic coast of Morocco in the south including the west and central region of the Mediterranean, at depths from 10 to 800 m (Chapman and Rice, 1971; dos Santos and Peliz, 2005; Figueiredo and Thomas, 1967). Over 30 different *N. norvegicus* populations are found in European waters and are fished wherever they are found in exploitable quantities (Bell *et al.*, 2006). As a result, the Norway lobster (referred to as *Nephrops* hereafter) is one of the most important commercial fishery resource in Europe (Bell *et al.*, 2006; Farmer, 1975; Sardà, 1998). *Nephrops* geographical distribution is highly discontinuous due to their dependent on particular types of seabed sediments. They have a preference for muddy seabed sediments, with >40% of silt and clay (Bell *et al.*, 2006). In Iceland, *Nephrops* are only found

off the southern part of the country on 10 discrete grounds at depths ranging between 100 and 300 m (Eiriksson, 1999).

Growth and population studies on *Nephrops* have always been restricted by the absence of direct age determination methods. As a consequence, historical methods used to assess *Nephrops* stock size are based on length-frequency data and rely on estimates of growth rates to establish mortality of the population. The stock assessment of *Nephrops* in Iceland is based on one of the most common methods to assess crustacean population abundance i.e. virtual population analysis (VPA). VPA uses fishery-depended data to determine the past stock size using mortality rates (Jennings *et al.*, 2001). Without reliable age-data, the VPA uses the von Bertalanffy growth parameters to convert length data into age classes (Ungfors *et al.*, 2013). This method has been criticised because of the lack of assumed variability in growth, and other external influences such as sampling and capture methodology (Ulmestrand, 2001; Sarda and Aguzzi, 2012).

As with other crustaceans, the growth of *Nephrops* is a discontinuous process consisting of a succession of moults or ecdyses and continues after the onset of sexual maturity without the occurrence of a terminal moult (Farmer, 1973; González-Gurriarán *et al.*, 1998). After the onset of sexual maturity however, the growth slows down considerably and moult frequency is 0–1 moults per year for females and 1–2 times per year for males with increments at moult typically between 1.0 and 2.5 mm CL (relative size increment of 3–12 %) but smaller and larger values have been seen in other *Nephrops* populations (Bell *et al.*, 2006). A relatively intensive tag-recapture study in Icelandic waters (recapture rate of 0.67%) recorded a mean growth per year of 3.5 mm CL (8.4 % increase) (Eiríksson, 1982a). Few tag-recapture studies have been reported on *Nephrops* and all have recapture rates below 6% (Chapman, 1982; Figueiredo, 1989; Ulmestrand, 2001). A major problem with tag-recapture studies is the low recapture rate for tagged *Nephrops*. The reason for low catchability is thought to be due to the burrowing behaviour of *Nephrops.* Recently, the feasibility of using the band counts as age indicator was investigated in few individuals of *Nephrops* in Ireland (Sheridan *et al.*, 2015) and in Iceland (Kilada *et al.*, 2015) but those studies did not validate or corroborate the annual deposition of the counted growth bands.
The objectives of this paper are (1) to corroborate observed band counts with previous age-length estimates; (2) to assess the repeatability of this method for *Nephrops* and estimate how practical it is for regular monitoring and (3) to compare the size-at-age relationships of the species in the two sites.

3.2 Material and methods

Nephrops were obtained from annual scientific *Nephrops* surveys in 2013 and 2014. Individuals were sexed and their body size measured (to the nearest 1 mm) as carapace length (CL, distance from the posterior rim of the eye socket to the dorsal posterior margin of the carapace) using vernier calipers. Males consist of more than 90% of catch landed in Icelandic *Nephrops* fisheries and were therefore only used in this study (Eiríksson, 1979).
A total of 110 individuals were collected from two of the largest fishing grounds in Iceland, Eldey (A) and Breiðamerkurdýpi (B) sites (55 males from each area) (Figure 3.1). Temperature recordings are not available for each site but since 1950 the MRI (Marine Research Institute of Iceland) have conducted annual hydrography observations in spring at a number of fixed positions on the Icelandic shelf in order to trace climatic variations. Temperature recordings from stations in close proximity to the two sites (Faxaflói and Ingólfshöfði) were obtained at depth of 100 m (Figure 3.1).

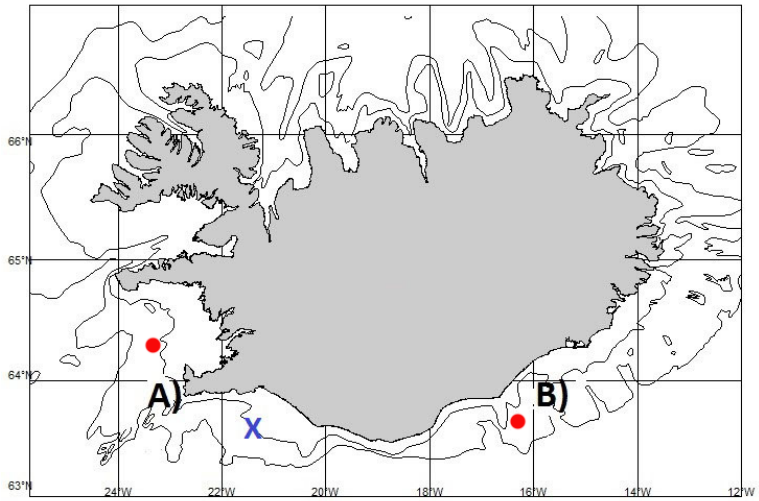

Figure 3.1. Sampling locations of Nephrops, Eldey (A) and Breiðamerkurdýpi (B). Blue X marks the location of the heaviest lobster documented in Iceland. Hydrography stations Ingólfshöfði (off the south coast) and Faxaflói are marked as red dots.

3.2.1 Protocol for age determination

All *Nephrops* specimens were frozen at sea and processed in a laboratory. Thin sections were prepared of all ossicles from the cardiac stomach; the zygocardiac, pterocardiac and mesocardiac ossicles. After removing the cardiac stomach, the ossicles were separated, cleaned of all organic tissue and stored in a solution of 4% glycerol, 26% water and 70% alcohol (Figure 3.2).

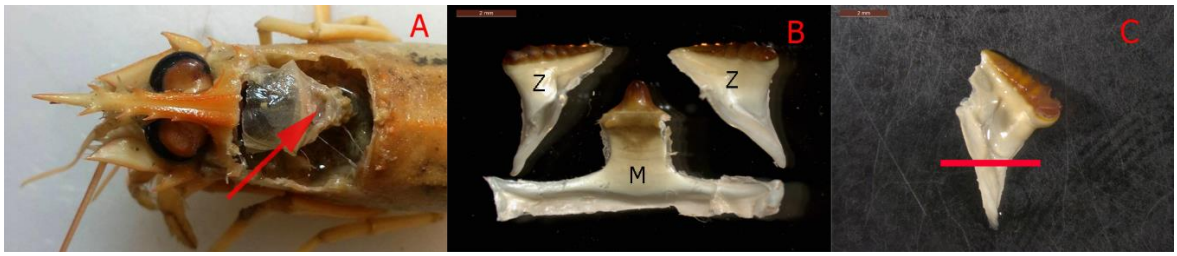

Figure 3.2. A: Male Nephrops specimen with exposed cardiac stomach and gastric mill (indicated by the black arrow) B: Nephrops gastric mill showing the structural arrangement of the single mesocardiac (M) ossicle and paired zygocardiac (Z) ossicle. C: Once cleaned, the zygocardiac ossicles were sectioned transversely approximately where the structure starts to narrow.

After cleaning, the structures were embedded in cold cure epoxy resin before preparing transverse sections of the zygocardiac ossicles (180-200 μm thickness) with a diamond-bladed precision saw (Buehler Isomet 1000). Sections were placed on a standard microscope slide and viewed with reflected light in 90% ethyl alcohol with a compound microscope (CX41 Olympus and Leica M165 C) at 100-400× magnification. Digital images were taken with a DP72 Olympus video camera attached to the microscope. Images were digitally enhanced using Adobe Photoshop 12.0.4 x32 to increase contrast between adjacent bands. Growth bands were recognized as paired light and dark zones in the endocuticle (Figure 3.3). They were counted independently by two readers from the basal (adjacent to the membranous layer and hypodermis) to the distal region of the endocuticle without knowledge of the animal's actual age or size-based estimate of age.

3.2.2 Statistical analysis

Coefficient of variation (CV) was calculated from the two readings to assess the precision of the band counts and reproducibility of repeated measurements. The CV was calculated as follows:

$$CV_j = 100 \times \frac{\sqrt{\sum_{i=1}^{R}(X_{ij} - \acute{X}_j)^2}}{\acute{X}_j}$$

where CV_j is the age precision estimate for the j^{th} individual. The CV values of all individuals are averaged across the whole sample to produce a mean CV, X_{ij} represents the i^{th} age of the j^{th} individual and $\acute{X}$ represents the average age of the j^{th} individual (Campana *et al.*, 1995). Potential growth rate differences between areas were assessed through an analysis of covariance (ANCOVA) considering areas as the covariant. All statistical analysis were performed using the computing environment R (R Development Core Team, 2015).

To compare the difference in growth between the two sites, an exponential growth model was fitted to the data. Further, the length-at-band count data was compared to age cohorts used in the routinely VPA analysis. There the "year-classes" are formed by slicing the

length-frequency distribution into knife-edged demarcations ranging from 6 mm (19 – 24 mm CL) to 2 mm (56+ mm CL) (Eiríksson, 1979). That was initially based on von Bertalanffy parameters derived from tagging experiments (Eiríksson, 1982a), examining length-frequency modes (Eiríksson, 1992) and examining the time it takes the average cohort to attain the next "year-class" (see Eiríksson, 1979).

3.3 Results

The bipartite growth bands in all sections consisted of a broad translucent zone bordered by a narrower opaque band (Figure 3.3 and 3.4). All counted growth bands were observed in the endocuticle layer of the cuticle and the first translucent zone (counting from the exocuticle) observed is far larger than the following bands. The zygocardiac ossicle was preferred over other ossicles as they had the clearest bands (see appendix). The band counts in this region were consistent between independent readers indicating no clear bias. The coefficient of variation (CV) values assessing the bias between the readings of independent readers using the zygocardiac ossicles were 6.3 and 7.2% in the *Nephrops* collected from site A and B respectively.

The current sampling included an unusually large individual (86.0 mm CL, 504 gr) from Selvogur (see location in Fig 3.1), which is indeed the heaviest *Nephrops* ever documented in Iceland. Of the 110 individuals collected, 51 depicted clear images for band counts. The number of growth bands ranged from 6 (35.6 mm CL) to 20 (82.2 mm CL) at site A and 7 (34.2 mm CL) to 13 (69.4 mm CL) at site B (Table 3.1). The large individual from Selvogur, expressed 22 bands (Figure 3.4). Using the growth band counts from the zygocardiac ossicles, a size-at-age relationships were created for both sites (Figure 3.5). In Eldey and Breiðamerkurdýpi, the size of 7 year old animals was proposed to be on average 42 mm and 41 mm CL, respectively. At Eldey, estimated 15 year old individuals where on average 76 mm CL.

According to the exponential growth model there was a significant difference (ANCOVA; $P = 0.004$) in growth rates between the two sites where individuals from site A grow faster (Figure 3.5). There was a good agreement with the estimated "year-classes" used in VPA analysis with estimated band counts from site B but at site A individuals estimated to be over 14 years of age where larger than expected compared to the VPA method (Figure 3.5).
Temperature data from 2000–2015 was gathered at 100 m depth from stations near the two sites. Average temperature in May at sites A and B was 7.0°C (SD = 0.275) and 7.8°C (SD = 0.274), respectively.

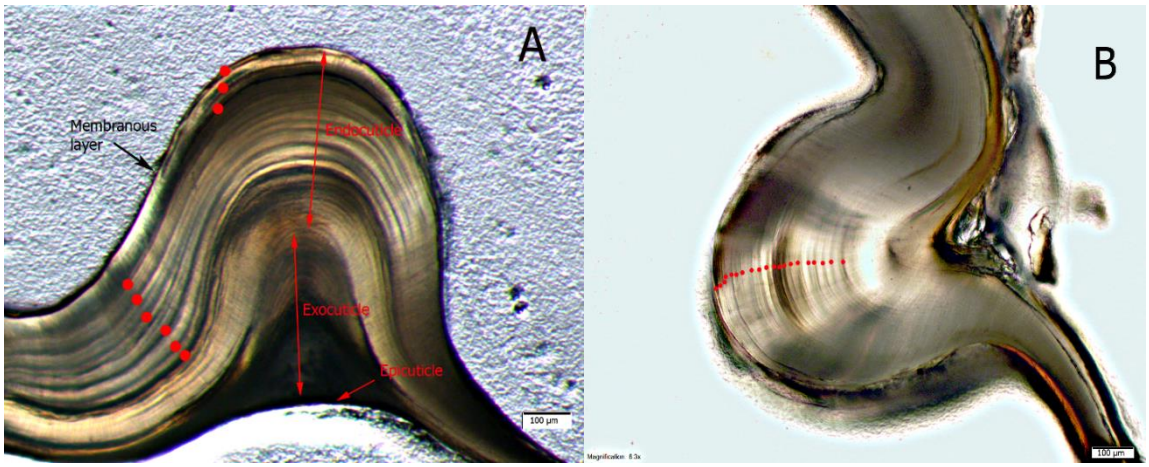

Figure 3.3. Growth bands indicated by red dots in a 200 µm thick transverse section of the zygocardiac ossicles from two Nephrops collected from Eldey-site (A: 46.6 mm and B: 82.2 mm CL).

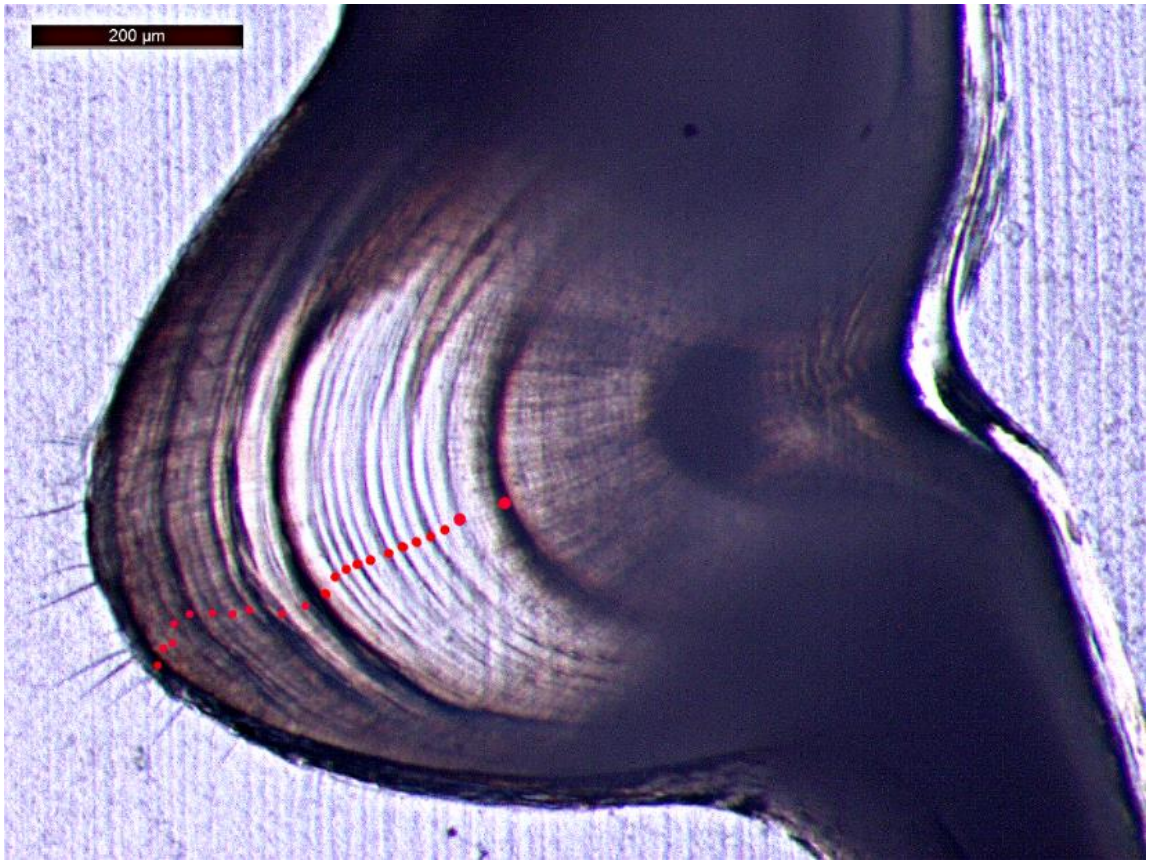

Figure 3.4. Largest Nephrops processed (86.0 mm CL) and estimated to be 22 years old. Growth bands are indicated with red dots. Thinner bands (lamellae) can be seen between the growth bands.

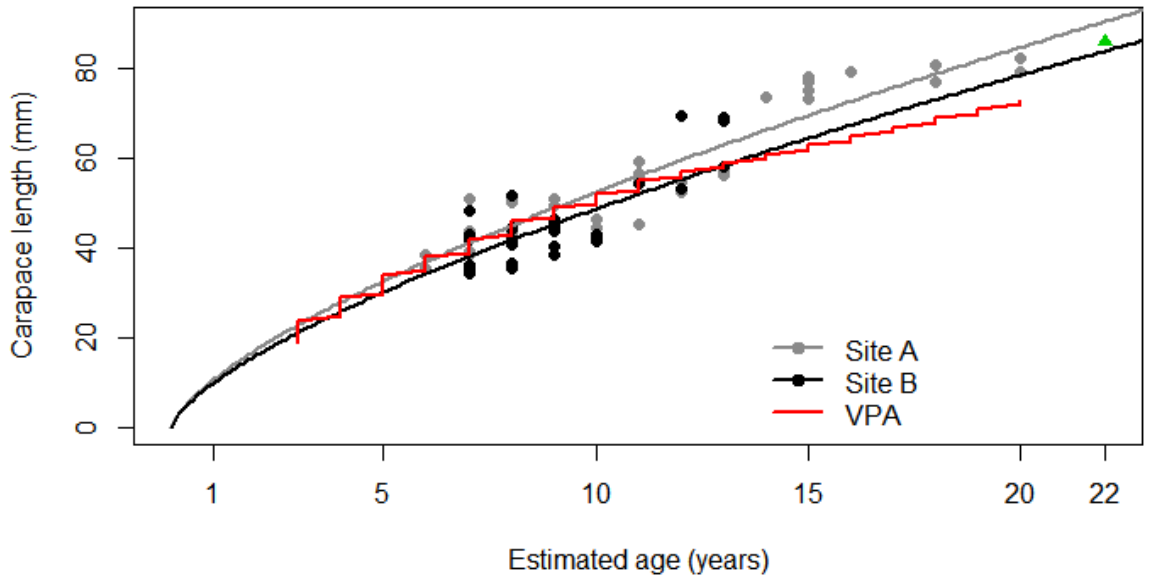

Figure 3.5. Size-at-age relationship estimated from growth band counts for Nephrops from Eldey (Site A) and Breiðamerkurdýpi (Site B). Lines for each region represent the fitted exponential growth equation. VPA line is the determined age from older length-frequency analysis (see text). The green triangle represents the heaviest Nephrops caught in Icelandic waters.

Table 3.1. Specimens of the processed Nephrops collected from Eldey (A) and Breiðamerkurdýpi (B) sites, Iceland. Historical values were gathered from annual MRI Nephrops surveys (2000-2015) and based on individuals measured in Eldey (n=5629) and Breiðamerkurdýpi (n=7108).

		Carapace length (mm)	**Band counts**	**Historical length values from surveys (mm)**
Eldey (Site A) *n* = 39	*Minimum*	35.4	6	23.0
	Maximum	82.2	20	82.2
	Mean	55.9	11.1	50.4
	SD	15.0	4.0	
Breiðamerkurdýpi (Site B) *n* = 32	*Minimum*	34.2	7	21.0
	Maximum	69.4	13	76.0
	Mean	46.6	8.9	42.9
	SD	9.9	1.9	

3.4 Discussion

In this study, Icelandic *Nephrops* was successfully aged using a novel technique based on growth bands in the gastric mill ossicle.
Growth bands were counted in the endocuticle layer of different gastric mill ossicles as previously documented for *Nephrops* (Kilada *et al.*, 2015; Sheridan *et al.*, 2015), the American lobster (*H. americanus*) (Kilada *et al.*, 2012) and several other crustaceans (Kilada and Acuña, 2015; Kilada and Ibrahim, 2016; Leland *et al.*, 2011, 2015; Tang *et al.*, 2015). All four cuticle layers where found in the gastric mill, the epicuticle, exocuticle, endocuticle and the membranous layer. As indicated by Leland *et al*. 2015, the age-zero ossicle must comprise of both exo- and endocuticle and therefore the boundary between them cannot be counted as the first growth mark. This was also observed in the *Nephrops* samples and gives more confidence to what we believe to be the first growth mark.

There was a lack of small individuals in this study which may be explained by two reasons; (1) the samples were obtained from the annual *Nephrops* trawl survey where the purpose was to estimate the stock size of fishable *Nephrops.* Therefore the same mesh size of 80 mm was used as in commercial fisheries and most individuals smaller than 35 mm CL were excluded from the trawl and (2) the recruitment numbers in Icelandic waters have been low and smaller individuals are getting extremely scarce (Anon, 2015). Further variations in length-composition of the *Nephrops* stock in Iceland has been observed over the last five years (Anon, 2015). Low recruitment numbers have been observed and the proportion of *Nephrops* larger than 70 mm is increasing (Anon, 2015). For future research it is strongly recommended to include smaller individuals.

For individuals under 50 mm CL, the growth band count was very similar to the previously estimated age. For larger *Nephrops* however, the band count differs from the estimated age. The estimated age is derived from length-frequency models which are best suited for young, fast growing animals. The longer a species lives, the methods becomes increasingly unreliable because young, fast growing individuals may group together with slowly growing individuals (Hartnoll, 2001; Vogt, 2012). Individuals expressing 9–10 growth bands were on average slightly smaller compared to those with 7–8 bands. These deviant year classes are hard to explain and can be caused by a number of factors apart from small sample size. The estimated stock size rose quite fast in the 2000s, reaching maximum around 2007–2009 (Anon, 2015). Growth of those year classes could have been impeded by density dependence effects but stock size fell quite rapidly from 2010. *Nephrops* have a long life span and the oldest observed *Nephrops* in this study was estimated to be 22 years old at CL of 86 mm (total length ~50 cm). There was no visible bias between two independent readers as the CV values for both sites were similar to those reported in other ageing studies for crustaceans (6-10%: Kilada *et al.*, 2012, 2015) and most fishes (5-12%: Campana and Thorrold, 2001).

The von Bertalanffy growth curve is often used to model growth in *Nephrops* (Bell *et al.*, 2006). For a successful modelling of a von Bertalanffy growth curve, all age groups need to be sufficiently represented in the samples. In our samples, we are missing representatives from the youngest age groups. Without the youngest individuals the means of the older age groups are spaced equidistantly and produce a linear growth relationship which can generate a large L_∞ value. There is also a gap of missing age classes in 60–70 mm CL range. Without these age classes, a bias accurse within the von Bertalanffy growth equation and causes a magnification of parameter estimation errors (Hillis, 1971; Smith *et al.*, 1997). Previous growth study on *Nephrops* in Icelandic waters estimated a *K* value of 0.101 and L_∞ to be 80 mm CL (Eiríksson, 1982a). Compared to other *Nephrops* populations, the Icelandic stock would be expected to have on average a lower growth coefficient than populations at lower latitudes because of lower water temperature. Off the Portuguese south and south-west coast, the *K* values for male *Nephrops* were found to range between 0.21–0.28 and L_∞ values between 61.5–71.5 CL (Figueiredo, 1984), while in the Clyde in western Scotland, they ranged between 0.16–0.22, and 45.5–65.5 mm CL (Tuck *et al.*, 1997).

Bottom temperature records are missing from the two sites but data from nearby stations (Figure 3.1) give a fair estimate of the temperature differences between the sites. The difference in temperature suggests that *Nephrops* from site B should grow at a faster rate. However, the factors influencing growth are highly complicated. Temperature, along with food supply and population density, is considered to be among the main influencing factor on growth (Hartnoll, 2001). The effect of low temperature on growth is that the time to reach certain stages of the life cycle (e.g. the onset of sexual maturity or the end of larval development) is increased, but the size when attaining that stage is also increased (Hartnoll, 2001). This tendency can be observed in different population of the same species that inhabit a wide range of geographical temperature gradients and leads to a general trend of smaller species in lower latitudes and larger species at higher latitudes. In Icelandic waters, ocean temperature has been elevating during the past decade (Valdimarsson *et al.*, 2012). Changes in ocean temperature surrounding Iceland has had a substantial impact on many other species. For example, the extension of southern commercial species further north (e.g. haddock, monkfish and mackerel) is most likely due to a warming of 1–2 C° in the waters south and west of Iceland during the past 15 years (Valdimarsson *et al.*, 2012). These changes could affect the *Nephrops* as well, but *Nephrops* has been found in more northerly locations in recent years (Jónas Páll Jónasson, pers. comm.).

Population density has also been identified as a key factor influencing growth (Tuck *et al.*, 1997). Where *Nephrops* are found in high densities, there seems to be a trade-off in the mean size (Ungfors *et al.*, 2013). The best density records are obtained using underwater television (UWTV) surveys to count *Nephrops* burrows and is a standard method to assess *Nephrops* populations in UK and Irish waters (Campbell *et al.*, 2008; Sarda and Aguzzi, 2012). No such survey has been performed in Icelandic waters. Even though population density records

are not available, density is speculated to be higher in site B. Therefore, despite of having higher temperature, site B also has higher population abundance of smaller individuals which limit *Nephrops* in that area from reaching greater size classes. Similar differences within *Nephrops* populations are not unheard-of and it has been documented that within the same biological population, there can be substantial variations in growth parameters and size structure (Hillis, 1971; Tully and Hillis, 1995). The exact reasons for the variability in growth rates are difficult to establish and are most likely combined effects of the aforementioned temperature and density but also several other driving forces (e.g. food availability, sediment particle size and fishing pressure) (Bell *et al.*, 2006).

The present study suggests that the new ageing method by counting annually deposited growth bands in hard structure of the gastric mill is applicable for age estimation of *Nephrops* in Icelandic waters. It is recommended that annual deposition of growth bands should be validated before implementing the technique into regular monitoring. Validation techniques using autofluorescent stains such as calcein can create identifiable marks in calcified hard parts. These techniques have shown promising results in the American lobster (*H. americanus*) and freshwater crayfish (*Cherax quadricarinatus*) (Kilada *et al.*, 2012; Leland *et al.*, 2015). Better knowledge in this field would not only improve our general understanding of *Nephrops* biology but if validated, it would have a substantial impact in fisheries management of this commercially important species.

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4 Appendix

4.1 Sample preparation and sectioning

Step 1 – Removal of gastric mill.
Nephrops carapace length (CL) is measured with a vernier calliper. The carapace is opened, the stomach removed and cleaned so only the hard structure of the gastric mill remains.

Figure 4.1. Position of the gastric mill in Nephrops.

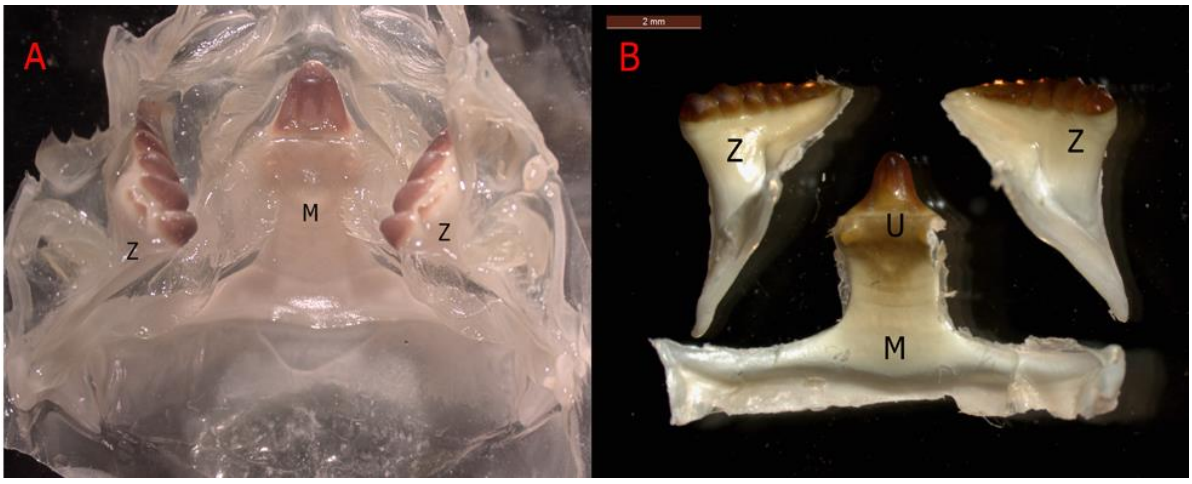

Figure 4.2. Gastric mill before and after cleaning.

Step 2 – Epoxy embedment and sectioning.

A pair of zygocardiac ossicles are placed into a plastic tray, the gastric teeth facing each other. Each tray is labelled before the epoxy is embedded with the appropriate name.

Epoxy is mixed, roughly 250 ml epoxy vs. 6-7 ml hardener and stirred gently in order not to form any air bubbles. The epoxy block is left to harden for 48 hours before it can be sectioned. In order to make a thin section of the gastric mill, a pair of sawblades with a thin plastic sheet (approximately 200 µm) seperating them is mounted on to the saw. The thin section is placed on a standard microscope glass and a drop of ethanol is put on the section to enhance the image before it is viewed in a microscope.

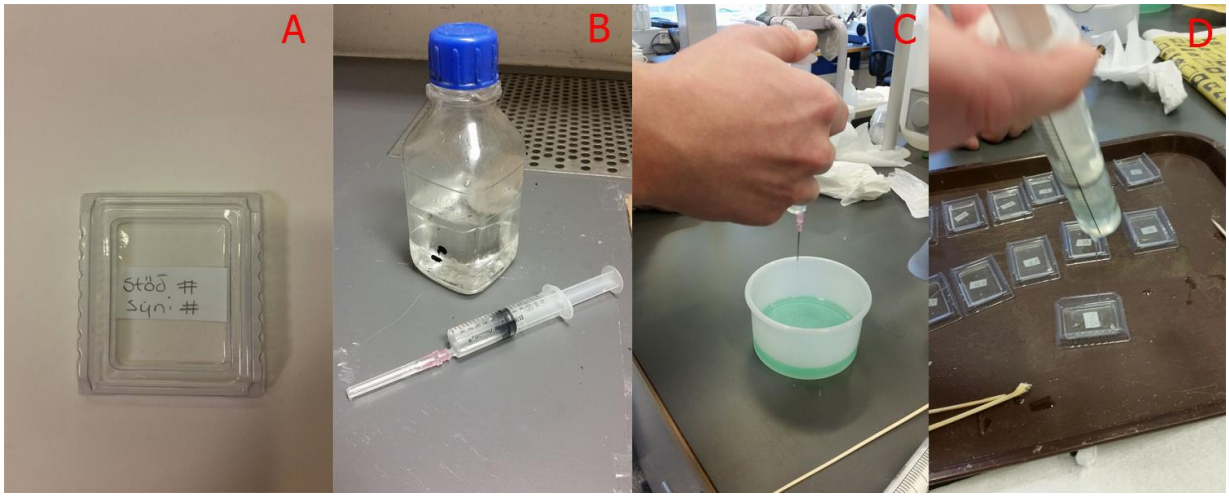

Figure 4.3. Preparation for moulding. A) Plastic tray/mould with a label. B) Hardener and syringe. C) Hardener mixed into epoxy. D) Epoxy injected into plastic moulds with a large syringe.

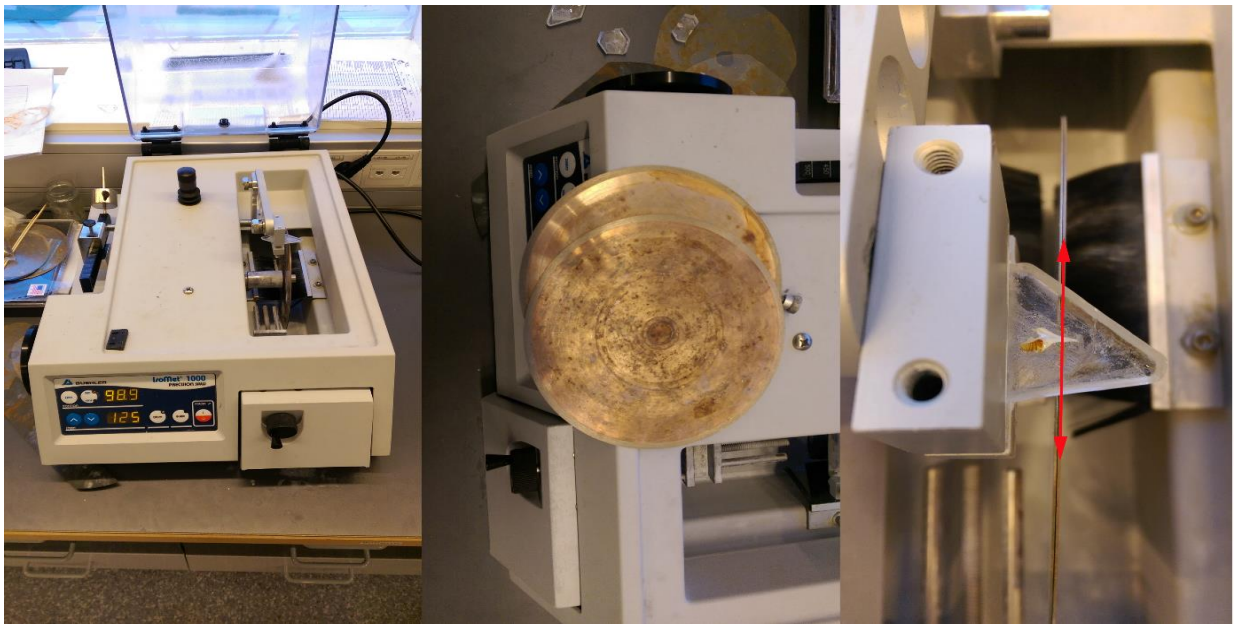

Figure 4.4. IsoMet™ 1000 Precision Sectioning Saw, pair of sawblades and an epoxy block mounted on the saw. The red arrow to the right indicates the cutting axis.

4.2 Growth band identification

The age determination method was applied to both mesocardiac and zygocardiac ossicles. The zygocardiac constantly produced clearer images, and therefore it was preferred in this study.

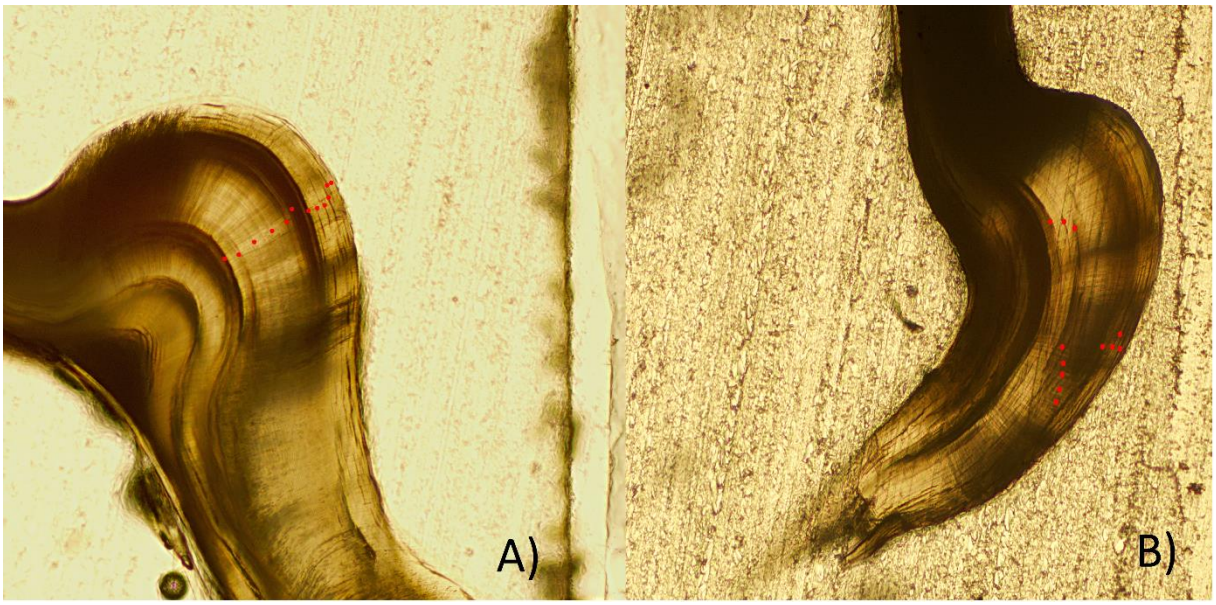

Figure 4.5. Zygocardiac (A) and mesocardiac (B) ossicles from the same individual (54.8 mm CL). Both images are enhanced in Photoshop to produce the clearest images. The red dots indicate 12 growth bands.

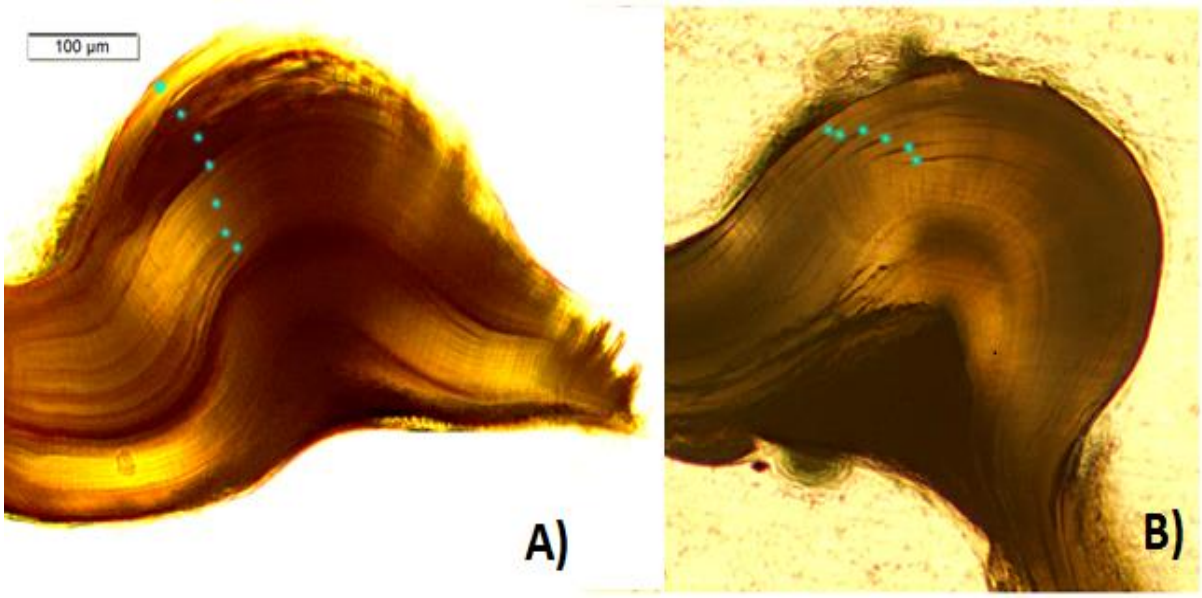

Figure 4.6. Zygocardiac ossicles from Nephrops with a carapace length (CL) of A) 46.63 mm (7 growth bands) and B) 35.43 mm (6 growth bands).

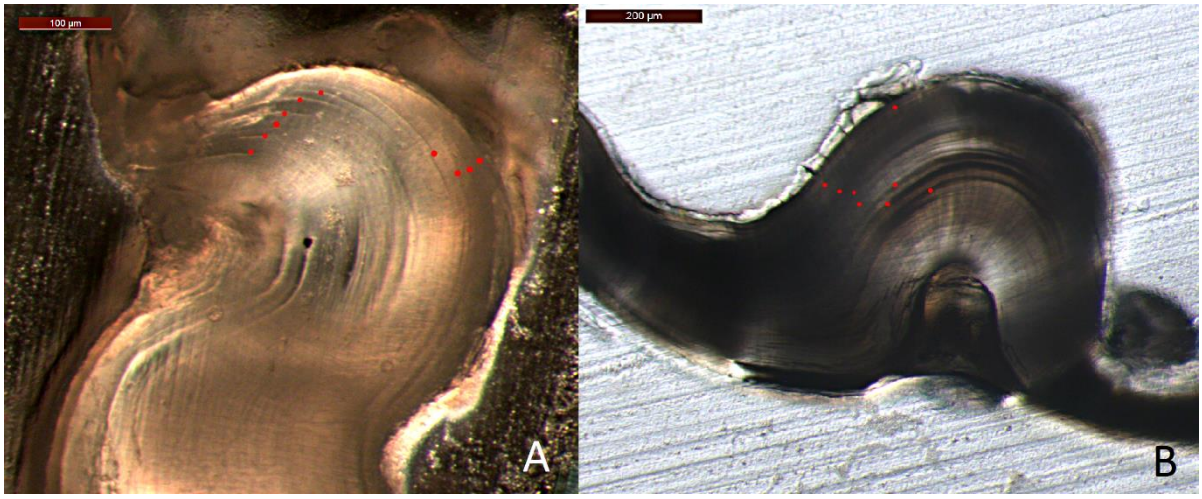

Figure 4.7. Zygocardiac ossicles from Nephrops with a carapace length (CL) of A) 46.1 mm (10 growth bands) and B) 44.1 (8 growth bands).

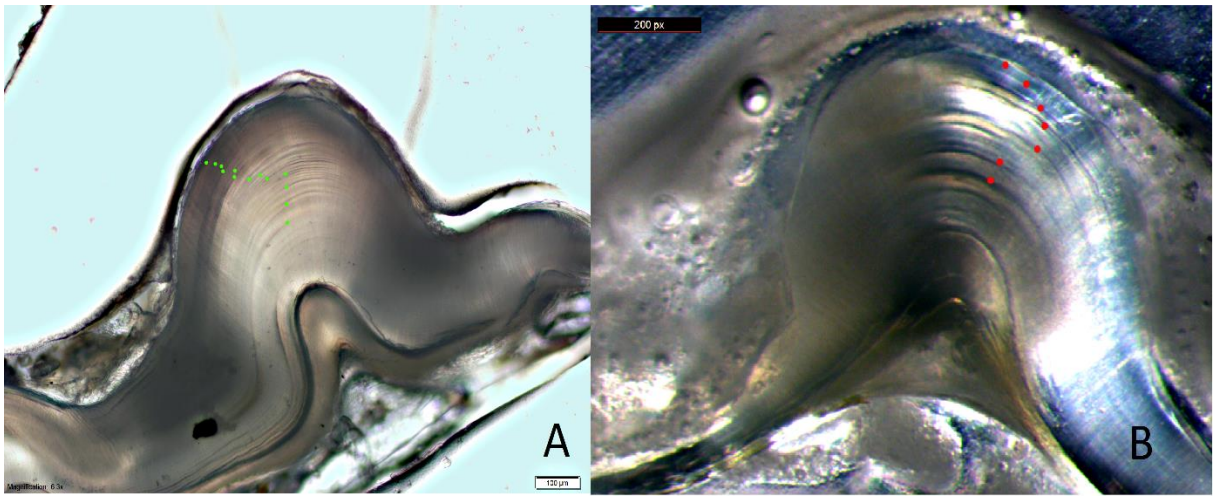

Figure 4.8. Zygocardiac ossicles from Nephrops with a carapace length (CL) of A) 69.3 mm (13 growth bands) and B) 35.1 mm (7 growth bands; one of the smallest Nephrops processed).

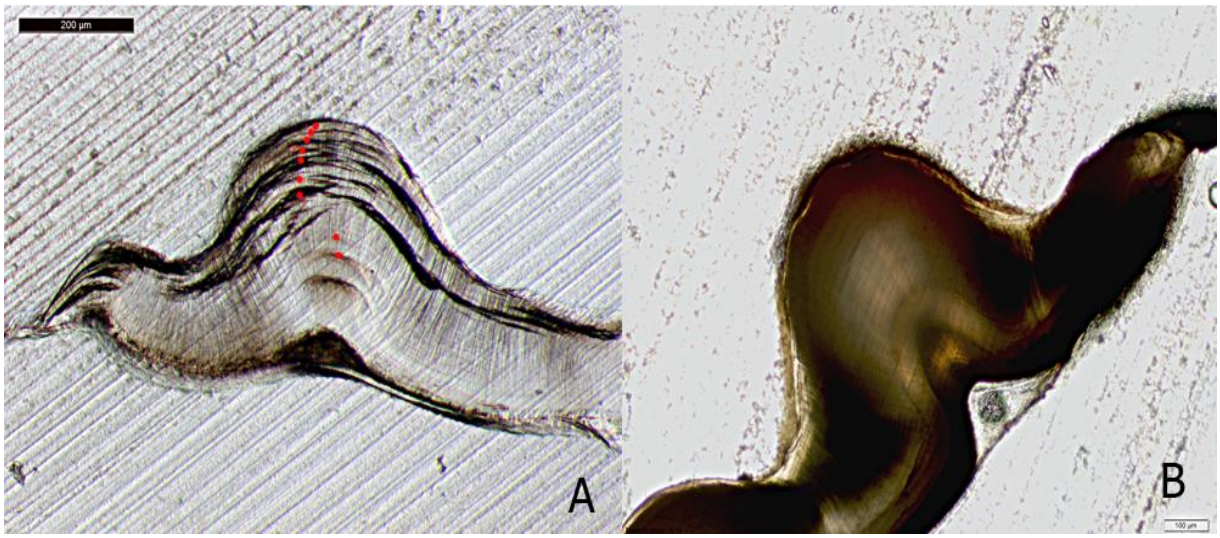

Figure 4.9. Examples of zygocardiac ossicle sections that are unreadable. Section A) is too thin (100 µm) and B) is too thick (400 µm).

4.3 Staining tests

When the growth bands were not clear (for example when the section was too thick) the use of Alizarin staining solution was tested in order to better identify the growth bands.

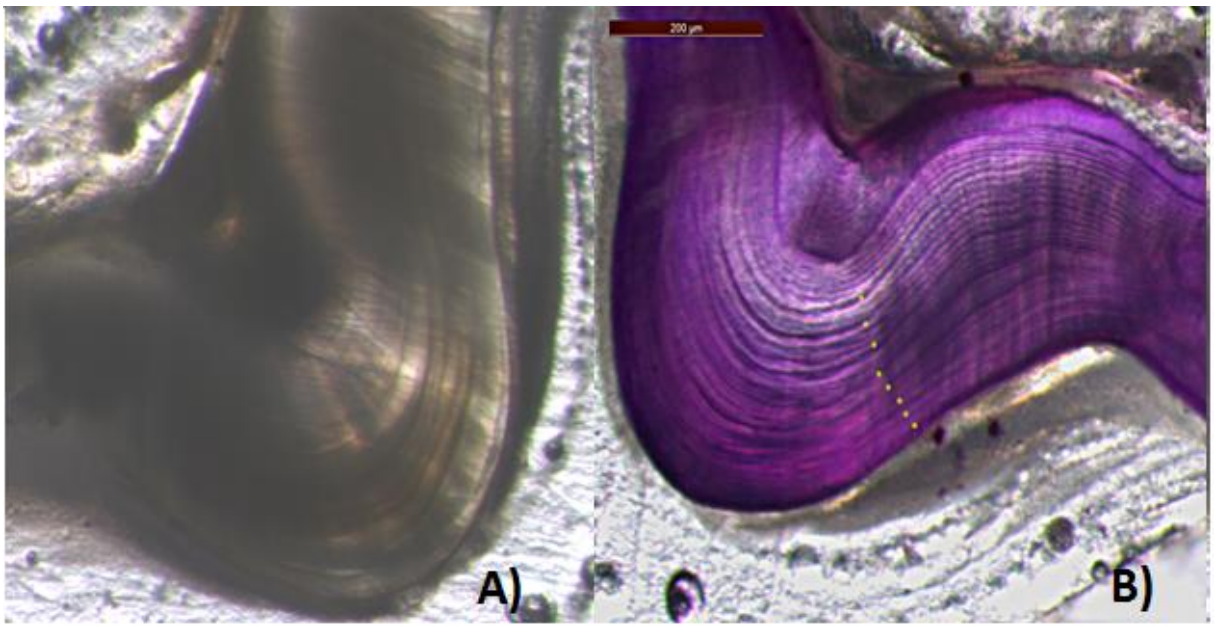

Figure 4.10. Zygocardiac ossicles from the same individual. The section on the right was placed in alizarin solution for 12 hours.

Alizarin stock solution (1.5L)
1) 6.2 g colour is mixed in 100 ml distilled water and 100 ml concentrated acetic acid and left to settle for approximately 20 min.

2) The mixture is added slowly to 300 ml glycerol and 1000 ml distilled water.

3) The solution can be stored in opaque, air tight container for up to 2 months.