Tools to examine population maturity and diet of *Nephrops norvegicus* on Irish fishing grounds

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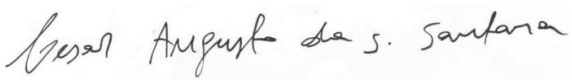
Declaration

I, Cesar Augusto da Silva Santana, certify that this thesis is all my own work and I have not obtained a degree in this University, or elsewhere, based on this work.

Signature: 

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Chapter 1

General Introduction

1. General Introduction

1.1. Lobsters

Lobsters are arthropods that make up the subphylum Crustacea, class Malacostraca and order Decapoda, along with crayfishes, crabs and shrimps (Hickman *et al.*, 2001). They are marine, crawling and elongated benthic organisms that are characterized by appendage-bearing 13-segmented cephalothorax (covered by an unsegmented carapace), plus 6-segmented abdomen, bearing 5 pairs of pleopods ('swimmerets'). Five pairs of walking legs or pereopods are attached to the thorax and it is this characteristic which gives the order its name from Greek, deca = ten and poda = feet (Hickman, 2001; Tshudy, 2013). In fact, the common name 'lobster' refers to a range of taxa that are not all closely related taxonomically, being classified into four separate infraorders: (i) Astacidea; (ii) Glypheidea; (iii) Achelata and (iv) Polychelida (Tshudy, 2013). The infraorders Glypheidea and Polychelida include, respectively, mostly extinct lobsters with claws or semi-chelated first pereopods, and blind lobsters with delicate and very elongated claws. Meanwhile, the infraorders Astacidea and Achelata comprise most of the extant and commercially important clawed and clawless lobsters (Hickman et al., 2001; Tshudy, 2013) e.g., the American lobster (*Homarus americanus*), the European lobster (*Homarus gammarus*), the Western rock (spiny) lobster (*Panulirus Cygnus*), Southern rock lobster (*Jasus edwardsii*), Caribbean lobster (*Panulirus argus*), Southern African west coast rock lobster (*Jasus lalandii*), Japanese lobster (*Panulirus japonicus*), and the Dublin Bay prawn (*Nephrops norvegicus*) that will be the subject of the present study.

1.2. Dublin Bay prawn

1.2.1. Taxonomic classification

The Dublin Bay prawn has a variety of common names across its geographical distribution e.g., Norway lobster (Norway); Langoustine (France) and Cigala (Spain) (Tshudy, 2013). The systematic classification of the Dublin Bay prawn is as follows: phylum Arthropoda Latreille,

1829; subphylum Crustacea Brunnich, 1772; class Malacostraca Latreille, 1802; order Decapoda Latreille, 1802; suborder Pleocyemata Burkenroad, 1963; infraorder Astacidea Latreille, 1802; superfamily Nephropoidea Dana, 1852; family Nephropidae Dana, 1852; genus *Nephrops* Holthuis, 1974; species *Nephrops norvegicus* Linnaeus, 1758. The genus *Nephrops* is monospecific, however there were an additional 13 species inside this genus until 1972, when these were moved to the genus *Metanephrops* (Tshudy, 2013). Besides the extant species *Nephrops norvegicus* (named as '*Cancer norvegicus'* by Linnaeus in 1758), a fossil species was placed in the genus in 2005: *Nephrops kvistgaardae*, with fossil record from the upper Miocene of Jutland, Denmark (Tshudy, 2013). Although *Nephrops norvegicus* has the typical morphology of other clawed lobsters, its body shape is slenderer and the claws are longer than in other clawed lobster such as *Homarus gammarus* (Hill, 2007).

1.2.2. Habitat and distribution

Nephrops norvegicus, hereafter called *Nephrops*, inhabits the continental shelf and slope of the northeast Atlantic, it is also found in the Mediterranean, Adriatic and Aegean Seas, as well as in Canary Islands (Figure 1.1) (Bell *et al.*, 2013; Johnson *et al.*, 2013). This species is found at depths from 20 to 800 m (Figure 1.1), however most of its populations are usually found at depths shallower than 200 m (Bell *et al.*, 2013). Along with favourable environmental conditions (temperatures between 6.4 – 17.3ºC; salinity between 31.8 – 38.8 and oxygen concentration between 5.9 – 9.4 mg O_2/dm^3), the existence of suitable sediment for burrow building is essential for *Nephrops* to colonize any area (Johnson *et al.*, 2013). A recent study about habitat suitability has associated higher *Nephrops* population density with higher content of silt and clay: between 60 and 80% (Lauria *et al.*, 2015).

1.2.3. Growth

As is the case for all arthropods, *Nephrops* grow by successive moults. Moulting is very frequent in juveniles (~ once per month), then the moulting frequency decreases gradually to 3

or 4 times per year around the second and third year of life, and after the onset of maturity, moulting occurs one or two times per year in males, and either one time per year or even less frequently in females (Farmer, 1973; Sardà, 1991; Bell *et al.*, 2013). It generally occurs in late winter, spring and late summer or autumn in males and in late winter or spring in females, after they have hatched the eggs (Farmer, 1973; Sardà, 1991; Bell *et al.*, 2013). Although there has been some research activity concerning age determination in crustaceans by the quantification of the concentration of the pigment lipofuscin in neural tissues (Bell *et al.*, 2013), currently, there is no feasible method for ageing *Nephrops* due to the lack of hard structures that record growth increments such as the otolith in fish, since crustaceans' hard structures are lost during the moulting process (Sheridan *et al.*, 2016). Despite this issue and the discontinuous growth in *Nephrops* by successive moults, the continuous von Bertalanffy growth function has been considered to be a convenient method to describe the relationship between age and growth in crustaceans for purposes of stock assessment and analysis of population dynamics (Bell *et al.*, 2013). *Nephrops* growth rates vary between and even within populations, possibly due to the combined effect of a variety of variables, e. g. temperature, sediment particle size, food availability, and fishing pressure (Bailey and Chapman, 1983; Bailey *et al.*, 1986; Tully and Hillis, 1995; Tuck *et al.*, 1997a; Bell *et al.*, 2013). Contrasting values for von Bertalanffy growth parameters can be observed across the range of *Nephrops'* geographical distribution, for example, mean asymptotic carapace lengths (L_∞) of 70.8 mm CL (males) and 55.2 mm CL (females) and respective growth constants (k) of 0.161 $years^{-1}$ and 0.077 $years^{-1}$ in the West of Ireland (Haynes *et al.*, 2016) against L_∞ = 94.86 mm CL, k = 0.41 $years^{-1}$ for males and L_∞ = 69.20 mm CL, k = 0.44 $years^{-1}$ for females in Portugal (Ayza *et al.*, 2011). In addition, growth in *Nephrops* is density-dependent with suppression of growth in high population densities (Johnson *et al.*, 2013; Merder *et al.*, 2019) and an inverse relationship between population density and L_∞ (Tuck *et al.*, 1997a; Johnson *et al.*, 2013). Density-dependent

processes (e.g. suppression of body size) has implications in the management of *Nephrops* stocks, since higher-density grounds may have more compensatory potential to counteract fishing (Ricker, 1954; Beverton and Holt, 1957; Merder *et al.*, 2019). Understanding these density-dependency issues is essential, for example, to determine the appropriate level of fishing exploitation across grounds of different densities or to decide whether there are implications for minimum landing sizes, especially if density-dependent processes impact on size at maturity.

1.2.4. Morphometry

Relative growth of various body structures has been an area of previous study in *Nephrops*, with these investigations including basic weight / length relationships, as well as the allometry of certain body structures at maturity. Allometry can be defined as a disproportional increase in size of any body structure of an individual relative to its body size (Bartels *et al.*, 2010). Allometric studies include relationships between the length of certain body structures and length of a 'reference' structure in the animal, generally the carapace length in *Nephrops* (Sardà, 1995). For example, Farmer (1974a) has shown changes in the growth rate of various *Nephrops*' structures which apparently take place at the onset of maturity, namely the female abdomen width, or propodus length of male claws, relative to the carapace length. These results were corroborated by McQuaid *et al.* (2006) who additionally showed allometric growth (at maturity) between appendix masculina and carapace length. The process above (allometric growth of body structures) is suggested to be related to a more successful courtship, mating and perpetuation of the species: larger abdomen in females, for example, indicates an increased capacity for carrying eggs and consequently increased number of offspring that will survive to adulthood. Similarly, larger claws in males are associated with successful courtship and mating, since larger and stronger claws increase the chance of success concerning antagonistic encounters in the search for a sexual partner (Tessier 1960; Farmer, 1974a; McQuaid *et al.*,

2006). This characteristic has been extensively used in the estimation of the size at the onset of maturity of *Nephrops* (see Farmer, 1974a; Hillis, 1981; Mori *et al.*, 1996; Tuck *et al.*, 2000; McQuaid *et al.*, 2006; Queirós *et al.*, 2013). Besides the body structures mentioned above (female abdomen width, propodus length of male claws and appendix masculina) a variety of other structures have been used for estimating the size at the onset of maturity (see Mori *et al.*, 1996). In Chapter 4 of this thesis, we propose a structure to be used for estimation of the size at the onset of maturity of males *Nephrops* for the first time: the first pleopod that is modified for copulation in this species (see Figure 1.3b). Morphometric relationships among body structures can also be a useful tool to characterize and discriminate distinct populations, whatever the factors (genetic or environmental) leading to such morphometric differences. Indeed, this tool has been used to characterize and discriminate stocks of a variety of aquatic resources (see Elliot *et al.*, 1995; Tzeng *et al.*, 2001; Paramo and Saint-Paul, 2010; Chen *et al.*, 2015; Siddiki *et al.*, 2016; Kalate *et al.*, 2018). Concerning *Nephrops*, there is only one study by Maynou and Sardà (1997) that morphometrically discriminates populations of two different areas of the Mediterranean Sea and associates such differences to environmental variables such as redox state and granulometry of the sediment. Chapter 4 of this thesis also proposed a density-dependent morphometric variability of *Nephrops* populations among a variety of Irish and Scottish grounds.

1.2.5. Reproduction

Nephrops are dioecious (separate sexes) with females and males distinguished by the position of gonopores (genital apertures, Figure 1.2), respectively, on the basal segments of the third and fifth pairs of pereopods (walking legs), as well as by the morphology of the first pair of pleopods (swimmerets): "robust and stout" in males and "slender, short and hair-like" in females (see Figure 1.3b,e) (Farmer, 1974b; Powell and Eriksson, 2013). Another structure exclusive to males is the appendix masculina (Figure 1.3c) on the second pair of male pleopods,

while the thelycum (cavity that accommodates the spermatophore after copulation, Figure 1.2b and 1.5), located between the fourth and fifth pairs of pereopods, is exclusive of females (Farmer, 1974b).

The male internal reproductive system is composed by the testis and the vasa diferentia (region where spermatophores are produced), while the internal reproductive organs of females includes the ovary and oviduct (Farmer, 1974c). The development of the female ovary can be described by sequential stages based on its colour and volume (Farmer, 1974c; Mente *et al.*, 2009). These developmental stages are summarized and illustrated in Table 1.1 and Figure 1.4, respectively, although a recent study has also considered the partial and full resorption of the ovaries for purposes of classifying female stages of maturation (see Becker et al., 2018, 2020 for details).

Female *Nephrops* generally present an annual reproductive cycle (Powell and Eriksson, 2013), however, a biennial cycle (each two years) is suggested for some regions, for example in the Mediterranean and North Seas (Sardà, 1991; Bianchini *et al.*, 1998). Indeed, Sardà (1991) emphasize that a biennial cycle (each two years) is possible as adaptation to local environments. The reproductive cycle in *Nephrops* is associated with water temperature, light intensity and photoperiod and thus varies across different geographical areas, depth and potentially according to annual weather patterns and climate (Farmer, 1974c; Bell *et al.*, 2013; Powell and Eriksson, 2013). Farmer (1974c) described the annual reproductive cycle of females *Nephrops* in the Irish Sea as follows: (i) eggs-incubation (September to April / May); (ii) eggs-hatching (April-June); (ii) moulting followed by copulation (May-August) and (iv) eggs-laying (August-September). The period of egg-incubation decreases with temperature (Farmer, 1974c). Thus, it is shorter in warmer regions at lower latitudes (Powell and Eriksson, 2013). For instance, the period of egg incubation lasts 6 months in the Mediterranean (eggs-hatching in December-March), but it is longer, at least 10 months, in Iceland (eggs-hatching in May-July) (Sarda,

1995, Powell and Eriksson, 2013). Furthermore, it is important to highlight that some studies in *Nephrops* suggest that berried females spent the whole period of egg incubation restricted to their burrows as evidenced by 'rare' observations of berried females in the catches during the winter (de Figueiredo and Thomas, 1967; Farmer, 1974c, Sardà, 1991). Copulation in *Nephrops* generally occurs at night around 24-48h after female moulting (Farmer, 1974c; Powell and Eriksson, 2013). The spermatophore is transferred to female thelycum (Figures 1.2b and 1.5) during penetration by the male where it is carried from copulation until next moult, when the eggs are fertilized when they pass through the thelycum's surface to be extruded underneath the abdomen (Figure 1.5) (Farmer, 1974c; Powell and Eriksson, 2013). *Nephrops* potential fecundity is exponentially related to the body size. For instance, the fecundities of females measuring 25 mm and 45 mm CL are circa 600 – 1200 and 3200-4800 oocytes respectively, however, effective fecundity is considerably lower than potential fecundity due to eggs loss (Bell *et al.*, 2013).

1.2.6. Size at the onset of maturity

Like the reproductive cycle, the size at maturity in *Nephrops* varies across different geographical areas (Sardà, 1995; Queirós *et al.*, 2013). Maturity in *Nephrops* can be assessed by different methodologies, e. g. direct observation of physiological characteristics, i.e. stage of maturation of the gonads (physiological maturity) or by investigating changes in allometric relationships, usually size of a particular structure 'x' relative to the standard length of the animal (usually the carapace length in *Nephrops*), identifying a statistically significant 'breakpoint' in that relationship, usually via partial regression (morphometric maturity). Studies on size at maturity across *Nephrops'* geographical distribution using different methodologies have estimated size at maturity in *Nephrops* ranging from 15.1 - 64.1 mm CL in males and 16 - 50.7 mm CL in females (de Figueiredo and Thomas, 1967; Farmer, 1974a; Mozizur, 1983; Bailey e*t al.*, 1986; Sardà, 1991; Bianchini *et al.*, 1998; Relini *et al.*, 1998;

Tuck *et al.*, 2000; McQuaid *et al.*, 2005; McQuaid *et al.*, 2006; Mente *et al.*, 2009; Ayza *et al.*, 2011; Queirós *et al.*, 2013; Bekrattou *et al.*, 2019). Size at maturity is important for stock assessment and management, since it plays an important role in the assessment of the stock spawning potential and the adoption of a minimum landing size. Despite the existence of some methods for estimation of size at maturity from physiological- to morphometrical-based methodologies (hereafter called SOM and MSOM, respectively), there are some issues concerning these methodologies. For example, a potential seasonal bias related to physiological-based methods which involve direct observation of gonads due to the seasonality of female reproductive cycle, as well as the need for expensive and time-consuming histological techniques to analyse internal structures of males (Lowerre-Barbieri *et al.*; 2011; Rotllant *et al.*, 2012). Another issue is the great variability of MSOM estimates, associated with different body structures as observed in Queirós *et al.* (2013) which prevents a reasonable choice of the real MSOM. In addition, Lowerre-Barbieri *et al.* (2011) argued about the efficiency of the methods above-mentioned and suggest the development of biochemical methods or improvements in analytical approaches and statistical techniques for estimation of the size at maturity. Observing this, in Chapter 3 of this thesis is proposed a new methodology for estimation of the size at the onset of maturity in females called the theoretical size at the onset of maturity (TSOM). The methodology is based on probability distributions of mature carapace lengths of *Nephrops* and theoretical probability distributions of immature female carapace lengths, created with information extracted from the probability distribution of the mature ones. Furthermore, TSOM was estimated for female *Nephrops* across a variety of Irish grounds. Finally, this chapter explored whether we can observe an inverse relationship between TSOM and population density, since as stated above, the understanding of density-dependent processes acting, e.g. on body size, are essential to the sustainable management of aquatic resources.

1.2.7. Feeding ecology

Nephrops are opportunistic predators and scavengers with a diet driven by prey abundance instead of preference (Bell *et al.*, 2013). The diet is similar across a wide geographical range and includes crustaceans, polychaetes, molluscs, echinoderms and fish (Chapman and Rice, 1971; Gual-Frau and Gallardo-Cabello, 1988; Cristo and Cartes, 1998; Parslow-Williams *et al.*, 2002; Bell *et al.*, 2013; Watts *et al.*, 2016). Bell *et al.* (2013) highlight that *Nephrops* feeding behaviour also includes cannibalism. Their foraging behaviour is connected with activity patterns, which are related to light intensity and consequently to depth range (Bell *et al.*, 2013). In shallow waters less than 30-40 m, peak emergence from the burrows is during the nocturnal period; at intermediate depths (40-100 m) emergence takes place at dawn and dusk and at depths > 100 m, emergence during the daytime can be observed (Bell *et al.*, 2013). One important issue to be addressed concerning the feeding ecology of *Nephrops* is about the mechanisms used by females to avoid starvation when they are restricted to the burrows during the breeding season (de Figueiredo and Thomas, 1967), since the existence of starvation in females during this period, e.g. in Scottish and Mediterranean grounds, has been refuted by the biophysical measure of the hepatosomatic index and the biochemical measures of hepatopancreas water, lipid and copper content, as well as biochemical analyses (proximal analyses and DNA/RNA) (Rotllant *et al.*, 2014; Watts *et al.*, 2016). In Chapter 1 of this thesis it is proposed that suspended particulate organic matter is an important item in *Nephrops*' diet and, in particular, that this is a feeding strategy which may be used for females to avoid starvation during the breeding season, as well as for smaller and more vulnerable individuals (including males) to avoid potential antagonistic encounters that may happen during the search for food.

1.2.8. Fisheries and management

Nephrops is exploited across its geographical distribution (Bell *et al.*, 2013). Fisheries for this species have increased over the last five decades in the northeast Atlantic and Mediterranean with landings increasing sharply and steadily until 1985 and stabilizing since then (Ungfors *et al.*, 2013). Landings reached 56,696 tonnes in 2017 (FAO, 2020), with United Kingdom and Ireland being the main producers with landings of 30,663 and 8,063 tonnes, respectively (FAO, 2020). From 2017 to 2019, Irish landings were on average 7,800 tonnes (Anon., 2020). Trawling is the main fishing method since *Nephrops*' habitat is suitable to this technique, however, static gears called 'creels' are important in some coastal areas in Scotland and Sweden, and this is the only fishing method used in the Faroe Islands (Bell *et al.*, 2013; Ungfors *et al.*, 2013). There are five main areas with active *Nephrops* fisheries: (i) the North Sea; (ii) Western Scotland; (iii) Celtic sea, Irish Sea and Western Ireland; (iv) Iberian Peninsula and (v) Mediterranean (Ungfors *et al.*, 2013). These areas have been separated in 30 discrete fishing grounds referred as 'functional units' (FUs; figure 1.6) for the purposes of stock assessment and 'management' (but see below) (Ungfors *et al.*, 2013). Irish Functional units assessed by The International Council for the Exploration of the Sea (ICES) include Eastern Irish Sea (FU14); Western Irish Sea (FU15); Porcupine Bank (FU16); Aran Grounds (FU17); Ireland SW and SE Coast (FU19); Labadie, Jones and Cockburn (FU20-21) and The Smalls (FU22). There is another functional unit: West of Ireland (FU18), however there is no major *Nephrops* fishery in this area and, thus, it is not considered for stock assessment and management (Ungfors *et al.*, 2013). An overview of the geographical location of ICES functional units can be seen in Figure 1.6, where Irish and Scottish functional units considered in Chapters 3 and 4 of this thesis are highlighted.

ICES is the organization which co-ordinates advice for the purposes of management of *Nephrops* stocks in the ICES member countries (European Union, Faroe Islands, Iceland and Norway). This is carried out according to a precautionary approach based on several

international agreements and policies (see Ungfors *et al.*, 2013; ICES, 2015). ICES advice considers also policies and legal needs of ICES member countries and multinational and intergovernmental organizations, responding to policies and legal instruments, such as 'The Common Fisheries Policy of the European Union', 'The Marine Strategy Framework Directive', 'The Act on the Management of Marine Resources', among others (ICES, 2015). Besides the policies and legal instruments mentioned above, recent policies that are slowly being incorporated into the process of management of *Nephrops* include 'ecosystem-based fisheries management', observing interactions among all components of the ecosystem, that is demanded by the Marine Strategy Framework Directive, and measures to reduce the practise of discarding, which is demanded by the 'Landings Obligation' within the reformed Common Fisheries Policy (CFP). As *Nephrops* is a 'non-quota species' it falls within a somewhat ambiguous 'management' regime, nevertheless, quotas or 'Total Allowable Catches' (TACs) are issued per FU by the statutory agency responsible in each ICES country (in Ireland, this is the Marine Institute). To support this exercise, the status and trends of *Nephrops* stocks are assessed annually for each Functional Unit (FU1-FU34) separately (with a few exceptions of adjacent FUs that are assessed together) – see Irish Stock Book 2020 (Anon., 2020). Following assessment, FUs are classified in different categories (1-6), which consider the quality and reliability of the data, and decrease from category 1 to category 6 stocks (ICES, 2015). Depending on the quality of the data, the assessment can be analytical (quantitative or qualitative treatment) or based on data-limited methods (ICES, 2015). The methods for assessing *Nephrops* stocks include length cohort analysis (LCA), virtual population analysis (VPA), production models and underwater television surveys (UWTV) (Bell *et al*., 2013; Ungfors *et al.*, 2013). UWTV assessments have shown that *Nephrops* abundance has fluctuated widely across Irish functional units since 2002, when UWTV assessment started in Irish waters (McGeady, 2020). Although abundance has been quite stable in the Western Irish Sea (FU15)

and Labadie, Jones and Cockburn (FU20-21) functional units, there has been considerable fluctuation in *Nephrops* abundance across the years in the other functional units (McGeady, 2020). For example, Porcupine Bank (FU16) was closed to fishing from 1 May – 31 July throughout 2010-2012 (closure reduced to May since 2013) or the Aran Grounds (FU17) where abundance estimates have declined significantly since 2004 and have been at or below the biomass at the maximum sustainable yield (MSY $B_{trigger}$) since 2012 (Anon., 2020).

1.2.9. Aims and objectives

The research presented within this thesis describes investigations on maturity and feeding ecology of *Nephrops* across some Irish and Scottish grounds in the Northeast Atlantic Ocean. This thesis aimed to address the gaps and ambiguities mentioned above, concerning the feeding ecology and the need for improvement in techniques related to the estimation of the size at maturity. The major objective of this study was to gain insights on the feeding ecology of *Nephrops*, as well as about the size at maturity of this species and, in particular, the effects of density-dependent processes on size at maturity and morphometry in *Nephrops* populations. These objectives were addressed using stable isotope analysis (SIA) in a Bayesian approach, multivariate morphometric techniques and a new methodology, developed in this study, for estimation of the size at the onset of maturity of female *Nephrops*, which might be particularly useful in data poor situations or for large-scale studies including macro-ecological comparisons.

The specific objectives addressed in this study were the following:

Chapter 2 – Importance of suspended particulate organic matter in the diet of *Nephrops norvegicus* (Linnaeus, 1758)

- to investigate the relative importance of suspended particulate organic matter in *Nephrops'* diet based on SIA according to a Bayesian approach;

- to examine seasonal sex-related differences in *Nephrops'* diet based on the SIA output;
- to examine size-related differences in *Nephrops'* diet based on the SIA output;
- to determine *Nephrops*' trophic position based on the SIA output.

Chapter 3 - Theoretical size at the onset of maturity and its density-dependent variability as an option in crustacean fisheries management

- to propose a new methodology for estimation of the size at the onset of maturity called the theoretical size at the onset of maturity (TSOM);
- to estimate the TSOM of female *Nephrops* in different Irish functional units;
- to test the hypotheses of an inverse relationship between TSOM and population density;
- to compare TSOM estimates to the existing measures including smallest berried female (SBF) and size at 50 % maturity (L_{50}, which represents the 'industrial standard' that is routinely used at present).

Chapter 4 - Morphometric size at the onset of maturity and discrimination of *Nephrops norvegicus* (Linnaeus, 1758) populations across a gradient of population density in the northeast Atlantic

- to examine a range of morphometric variables that may indicate onset of maturity (termed 'MSOM') across Irish and Scottish grounds, based on different body structures in male and female *Nephrops,* including one structure used for the first time for this purpose, the male first pleopod that is modified for copulation;
- to verify a potential inverse relationship between the 'best' morphometrical maturity metrics and population density across the grounds in the study;
- to examine possible morphometric discrimination of *Nephrops* populations at a variety of Irish and Scottish functional units;

- to identify the main characters contributing to the discrimination of *Nephrops* populations at a variety of Irish and Scottish functional units;
- to investigate any link between morphometric discrimination of *Nephrops* populations and population density across the grounds in the study

1.3. Tables

Table 1.1. Overview of ovary development stages in female *Nephrops* (from Powell and Eriksson, 2013)

Stage	Status of female	Ovary description
0	Not yet sexually mature or have undergone resorption postmoult.	White and threadlike (1–1.5 cm). Previtellogenic.
1	Initial oocyte development.	Cream-coloured (2 cm). Early vitellogenic.
2	Intermediate oocyte development.	Pale green just visible through carapace (2.5 cm). Medium vitellogenic.
3-4	Maximum oocyte development, stages could be split according to size of ovary.	Dark green, visible through back of carapace (3–3.5 cm). Late vitellogenic.
5	Almost spent. (Additional regeneration stage.)	Mottled green/cream, similar in size to stage 1 ovary.

1.4. Figures

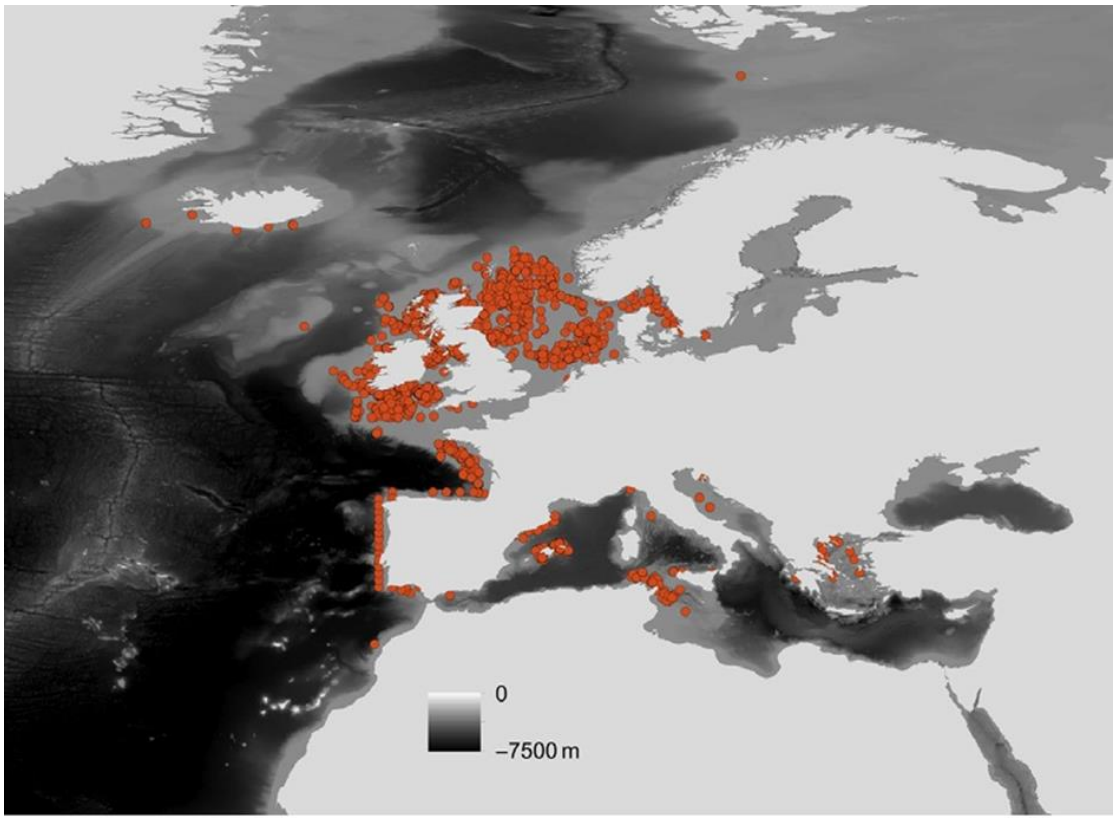

Figure 1.1. Geographical distribution of *Nephrops*, red points refers to species records according to the Ocean Biodiversity Information System (OBIS) database. Figure taken from Johnson *et al.* (2013).

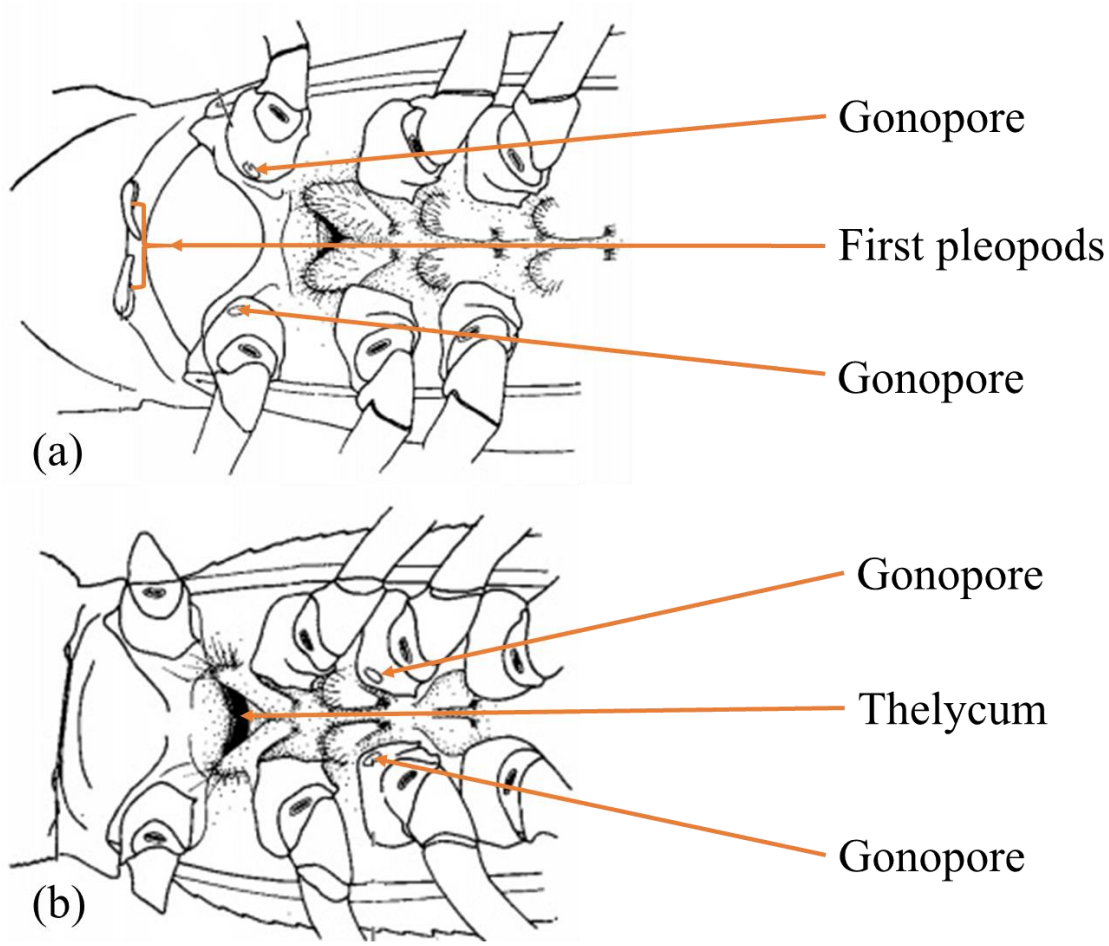

Figure 1.2. Ventral view of the posterior thoracic segments of: (a) male *Nephrops norvegicus* showing the genital aperture (gonopore) in the basal segments of the fifth pair of pereopods (walking legs) and the first pair of pleopods (swimmerets) modified for copulation; (b) female *Nephrops norvegicus* showing the genital aperture (gonopore) in the basal segments of the third pair of pereopods and thelycum. Figure taken and adapted from Farmer (1974b).

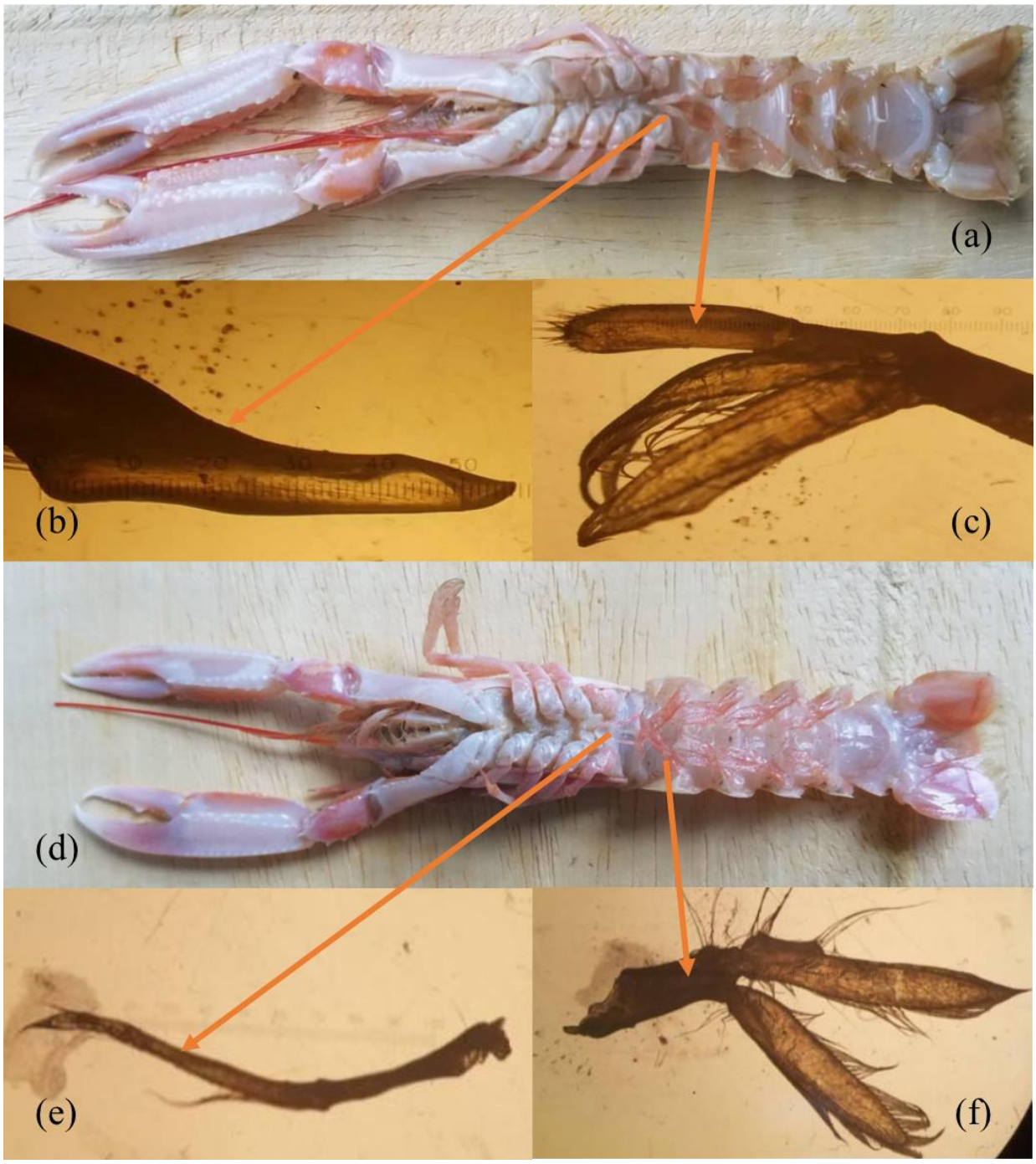

Figure 1.3. Ventral view of *Nephrops norvegicus*: (a) male *Nephrops*; (b) male first pleopod (swimmerets) modified for copulation (10x magnification); (c) modified male second pleopod with appendix masculina (10x magnification); (d) female *Nephrops*; (e) female first pleopod (10x magnification) and (f) female second pleopod (10x magnification). Photo credits: Conor Smyth.

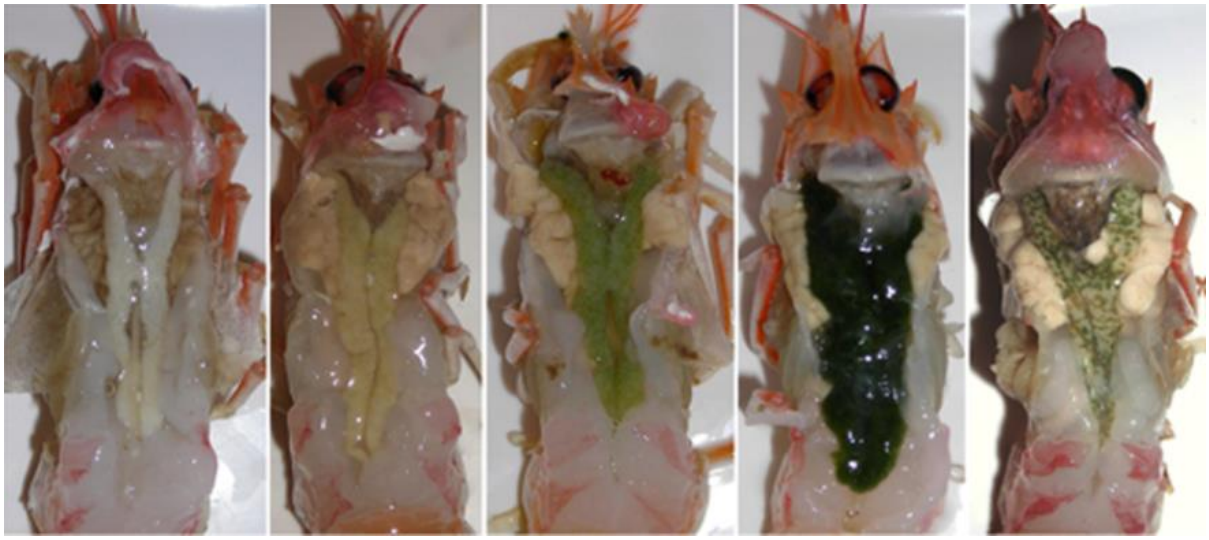

Figure 1.4. Ovary stages (see Table 1.1) in mature female *Nephrops* (figure taken from Powell and Eriksson, 2013).

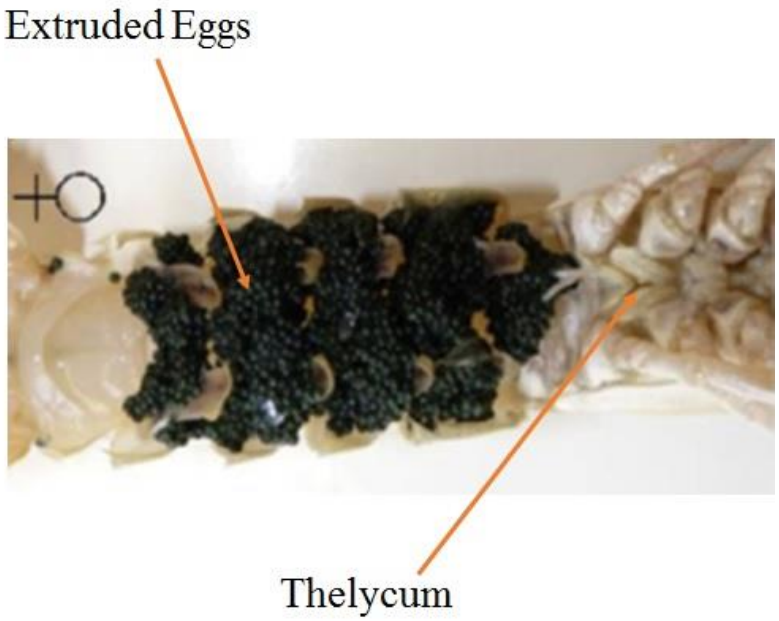

Figure 1.5. Ventral view of female *Nephrops* with extruded eggs underneath the abdomen, after they pass through the thelycum surface (figure taken from Queirós *et al.*, 2013)

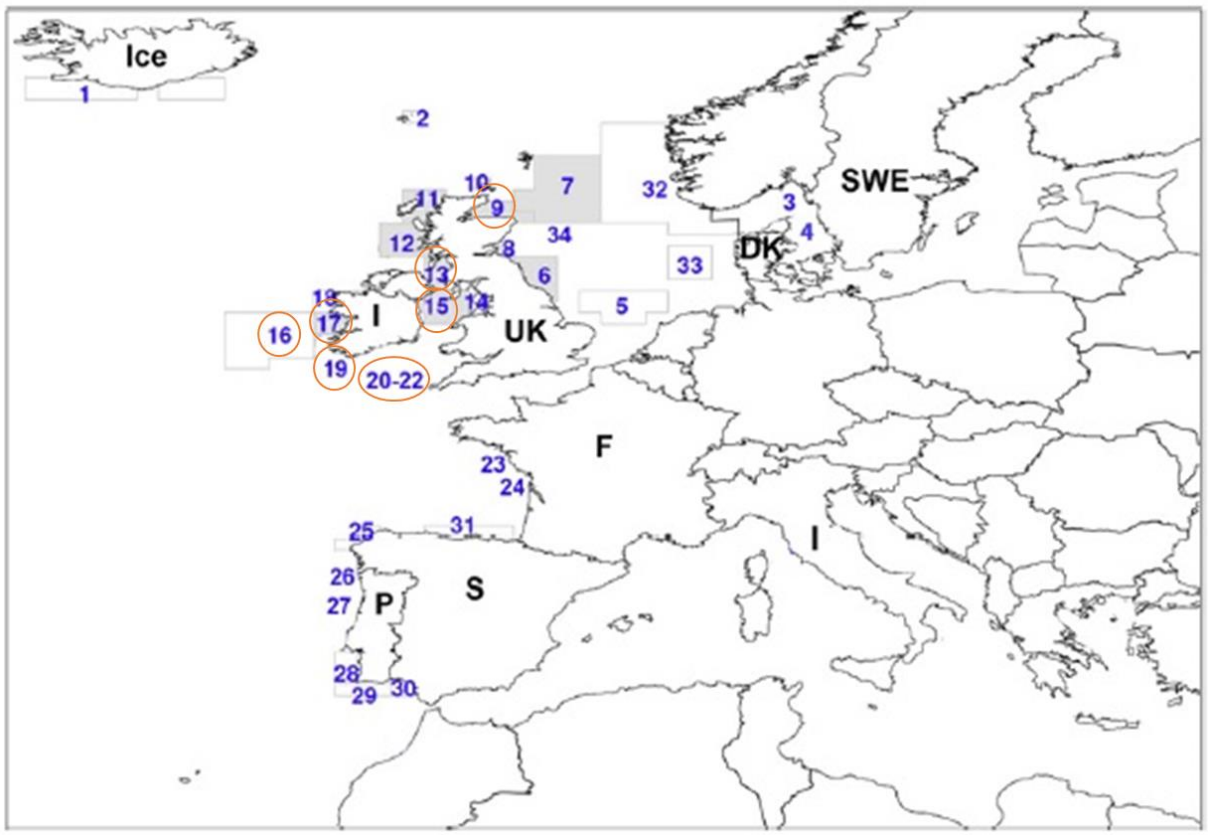

Figure 1.6. Geographical location of ICES functional units (FU1-34). Highlighted in orange are functional units considered in the studies described in chapters 3 and 4 of the thesis: Moray Firth (FU9); Firth of Clyde (FU13); Western Irish Sea (FU15); Porcupine Bank (FU16); Aran Grounds (FU17); South Coast (FU19); Labadie, Jones and Cockburn (FU20-21) and The Smalls (FU22). Figure taken and adapted from Ungfors *et al.* (2013).

Chapter 2

Tools to examine *Nephrops*' diet

2. Importance of suspended particulate organic matter in the diet of *Nephrops norvegicus* (Linnaeus, 1758)

Except for trophic position estimates (improved herein), the results of this chapter has been published as a peer-reviewed publication: Santana, C. A. S., Wieczorek, A. M., Browne, P., Graham, C. T. and Power, A. M. 2020. Importance of suspended particulate organic matter in the diet of *Nephrops norvegicus* (Linnaeus, 1758). Sci Rep 10, 3387. https://doi.org/10.1038/s41598-020-60367-x

Author contributions: A.M.P., C.A.S.S. and C.T.G. designed the study and carried out the statistical analysis; A.M.P. and C.A.S.S. wrote the manuscript, prepared the figures and reviewed the manuscript. C.T.G., A.M.W. and P.B. carried out the fieldwork, prepared the samples and reviewed the manuscript. A.M.P. and C.A.S.S. obtained the funding.

Abstract

The extent to which commercially important *Nephrops norvegicus* lobsters feed on particulates in the wild is unknown, even though this could be an important way for burrow-dwelling females to avoid starvation during the long breeding season. This was investigated using $\delta^{13}C$ and $\delta^{15}N$ stable isotope values in tissues with long and short turnover rates to provide diet discrimination and compare this between males and females. Secondary objectives examined size-related differences and calculated the trophic position based on the new results. Our estimates indicated that almost half the diet (47%) was made up of suspended particulate organic matter (POM_{susp}) alone. Fish was another important item in the diet, with plankton and invertebrate sources coming much lower down in dietary importance. Significantly more suspension feeding was observed in small or medium sized individuals than large ones in both sexes. However, there were no sex-related patterns, despite females being restricted to burrows for part of the analysis period. Female diet was almost identical to males and POM_{susp} comprised a large component of the diet in both sexes. The trophic position was estimated at

3.50, which was around the middle point of the range reported in previous studies (2.60 to 4.32).

2.1. Introduction

Dublin Bay prawn, *Nephrops norvegicus*, is a decapod crustacean and an important economic resource in Europe: global production of this fishery was 59,033 tons in 2016 of which the United Kingdom and Republic of Ireland were the main producers, capturing up to 32,708 and 10,379 tons per annum respectively during 2012-2016 (FAO, 2016). *Nephrops* populations are distributed on semi-isolated mud patches which are assessed by ICES as separate Functional Units (FU), however this resource is not 'managed' via fishing quotas, and some FUs periodically display signs of over-exploitation (Anon., 2011; Lordan *et al.*, 2013). The Marine Strategy Framework Directive and reformed Common Fisheries Policy (CFP) require ecosystem-based fisheries management which observes interactions among all components of the ecosystem, including trophic interactions (Hobday *et al.*, 2011; Johnson *et al.*, 2013; Möllmann *et al.*, 2014). Although not currently managed under the CFP, key gaps and ambiguities exist in knowledge of *Nephrops'* diet and feeding ecology which should be addressed, given their economic importance and occasional over-exploitation.

Nephrops individuals are known to be opportunistic predators and scavengers, which seem to have a diet driven by prey abundance rather than prey preference (Chapman and Rice, 1971; Parslow-Williams *et al.*, 2002; Bell *et al.*, 2013; Watts *et al.*, 2016). Diet from stomach contents analyses seem to be similar across a wide geographical range in the north-eastern Atlantic and Mediterranean, composed mainly of crustaceans, polychaetes, molluscs and echinoderms (Parslow-Williams *et al.*, 2002; Bell *et al.*, 2013). A considerable contribution to the diet is also made by fish in southern Atlantic and Mediterranean samples (Gual-Frau and Gallardo-Cabello, 1988; Cristo and Cartes, 1998). However, some mystery surrounds the extent of feeding on particulates in *Nephrops*. In the absence of alternative food sources, such as in the

aquarium, *Nephrops* was demonstrated to feed on planktonic items larger than 300-500 µm, which were later recovered from the stomach and intestine of the animal (Loo *et al.*, 1993). But, although some studies use particulate organic matter (POM) as the baseline for *Nephrops* trophic position estimation (Loc'h and Hily, 2005; Watts, 2012), no studies have directly measured the importance of particulate food items in the diet of *Nephrops* in its natural habitat.

The lifestyle of male and female *Nephrops* differs significantly as females are restricted to burrows while brooding embryos over a long breeding period, from six to ten months, depending on latitude (Powell and Eriksson, 2013). Therefore, it is logical to ask whether there are sex-related differences in diet arising from these lifestyle differences. Restriction to burrows for most of the year is evidenced by a lower percentage of females in fisheries catches during the breeding season, which is extended in Irish and Scottish grounds over autumn, winter and early spring (de Figueiredo and Thomas, 1967). For this reason, a seasonal decrease in nutritional status, i.e. 'starvation', has been predicted for females in winter, compared with late Spring and Summer when they are observed to be present in the catch and actively feeding after releasing their broods, moulting and mating (Sardà, 1991, 1995; Johnson *et al.*, 2013; Watts *et al.*, 2016; Powell and Eriksson, 2013). A biochemical index for estimation of nutritional status of females from the Clyde Sea in Scotland suggested that, although females had reduced nutritional status in the winter, this was not sufficiently low to indicate starvation (Watts *et al.*, 2016). At the same time, growth in females is also much lower than in males (Haynes *et al.*, 2016) and the respective diets of males and females are still not fully understood.

Suspension feeding has been identified as a possible strategy for females to survive starvation while they are restricted to burrows during the long breeding period (Loo *et al.*, 1993). Since suspended food in the form of plankton biomass is seasonally lower during the winter female burrow-dwelling period (Pinet, 2003; O'Boyle and Silke, 2010), we propose that females instead feed on suspended POM (i.e. POM_{susp}). POM_{susp} represents a complex microscopic

mixture of living and non-living organisms including phytoplankton, fecal pellets, detritus, bacteria and heterotrophs, but with distinct isotopic values from phyto- and zooplankton, particularly in coastal areas (Harmelin-Vivien, 2008; Stowasser *et al.*, 2012), that can be an important food source to many organisms. The main aim of this study was therefore to investigate the relative importance of POM_{susp} in *Nephrops*' diet (all sexes) as well as to examine sex-related differences in diet during the Spring-Summer period. Secondary objectives were to compare the diet composition between adult size classes as we might expect some dietary differences between the smaller and larger sizes due to differing abilities to compete over prey or to handle different prey items. Stable isotopes analysis (SIA) was chosen to complement information from previous stomach contents analyses (Gual-Frau and Gallardo-Cabello, 1988; Cristo and Cartes, 1998; Parslow-Williams *et al.*, 2002; Bell *et al.*, 2013). SIA can more fully represent POM_{susp} and soft-bodied prey items in the diet as well as providing a time-integrated view of feeding compared with a 'snapshot' provided by stomach contents analysis (Wieczorek *et al.*, 2018). SIA analysis on tissues with different turnover rates can also demonstrate diet compositions over distinct periods (Fry, 2006). For example, in the present study, $\delta^{13}C$ and δ^{15} N stable isotope values in long and short-term storage tissues were used to compare signals in *Nephrops'* diet between males and females, both in the period when females were in burrows during the lead-up to spawning and the period after females had spawned and were actively feeding, maturing new gonads and mating. A final aim was to determine *Nephrops*' trophic position based on new SIA results from the present study. For purposes of diet discrimination and hypotheses testing, the SIA data were analysed within a Bayesian framework, an approach which is increasingly used to address ecological problems (Ben-David *et al.*, 1997; Carrasco and Perissinotto, 2010; Fanelli *et al.*, 2011; Negrete *et al.*, 2016; Segura-García *et al.*, 2016; Villegas *et al.*, 2016; Bosley *et al.*, 2017; Herman *et al.*, 2017).

This study examined the importance of POM_{susp} as a food source in the diet of wild *Nephrops*, comparing this with other food sources. The specific hypotheses tested were: i) suspension feeding is higher among the smaller (more vulnerable) adults, either because they remain in burrows to avoid enemies, or because they are too small to handle larger more mobile prey; and ii) feeding patterns are sex-related, specifically there is higher suspension feeding in females than males during the period when females are brooding embryos and restricted to burrows compared with the period post-spawning when they are actively moulting, mating and feeding outside of the burrow.

2.2. Methods

2.2.1. Study area

The research was conducted on an inshore population at Clew Bay in the west of Ireland. Samples were collected in Clew Bay (53.87˚N and 9.64˚W) on substrates which are dominated by mud and sand and in water depths ranging from 5–40 m (average 20 m). The tidal range is around 5 m and the residence time of water in the inner bay is likely to be short, ~2 days (Anon., 1999, 2001). Pot fishing for *Nephrops* is seasonal in this area and runs from April – August, therefore the field sampling programme was limited to this period.

2.2.2. Sample collection

All samples including *Nephrops* and putative prey (benthic macrofauna, zooplankton, phytoplankton and suspended particulate organic matter), were collected from the study site on two occasions which were eight weeks apart, i.e. on the 29-May-2014 (nominated *'Spring long'* or *'Spring short'*, depending on the tissue – see below and Table 2.1) and the 25-July-2014 (*'Summer long'* or *'Summer short'* – Table 2.1). *Nephrops* were collected by baited creels on both dates. As *Nephrops* interacts with benthic communities both on and beneath the sediment, these potential prey items were obtained in two ways: (1) via five 15 min bottom trawl, using

a standard 2 m beam trawl with a chain mat, a stretched mesh (bar length: 20 mm) and a codend liner with a knotless mesh (bar length: 4 mm) and (2) using a day grab Van Veen 12.110 - 250 cm^2 / 3.14 L. A total of 17 different putative prey taxa were sampled from different groups: tunicates, polychaetes, bivalves, gastropods, crustaceans and fish on both dates (see Supplementary Table A2.1 in appendix 1). Zooplankton and phytoplankton were sampled on both days using a 57 cm ring diameter and 250 µm mesh WP-2 plankton net towed behind the boat (15 min tows). The choice of plankton net assumed *Nephrops* can feed on plankton items larger than 300-500 µm (Loo *et al.*, 1993). Assuming that *Nephrops* consumers fell within the range of diet sources in an isotope bi-plot, we could be satisfied that no important food sources were missing from the analysis (indeed, this was the case - see Results section 2.3). To sample POM_{susp}, water samples were taken via a Niskin bottle triggered at around 1 m above the seafloor. All samples were held on ice during transit and then transferred to a -20˚C freezer until processing for stable isotope analysis.

2.2.3. Stable isotope sample preparation

Potential *Nephrops* food 'source' tissues were processed as follows: phytoplankton and zooplankton samples were cleaned under the microscope. POM_{susp} was concentrated by filtering seawater (around 5 L was filtered for each sample) on precombusted glass filters and stored frozen (-20˚C). POM_{susp} samples were acid-washed to remove any carbonates, which consisted of adding 1 ml 0.1M HCl, following the protocol developed by Jacob *et al.* (2005). All macrofaunal items that were dominant in both abundance and biomass in grabs were sampled for SIA using various tissues, depending on the organism (see Supplementary Table A2.1 in appendix 1 for details).

'Consumer' (*Nephrops*) tissues were subsampled from the fisheries catch by selecting n=10 replicate individuals within each of three size classes (small, medium and large) for both sexes (see Supplementary Table A2.2 in appendix 1). After thawing at room temperature, carapace

length, weight without chelipeds (to avoid bias due to claw loss) and sex was recorded for all individuals. *Nephrops* tissue was sampled from muscle (tail) and hepatopancreas for both males and females, with hepatopancreas in this case representing a shorter-term storage tissue and muscle representing a longer-term storage tissue (see below).

All tissues sampled were oven dried in 2 ml tubes at 60˚C for at least 48 h. Each dried sample was then ground with a mortar and pestle to a fine homogenous powder. Varying amounts of lipids amongst species and tissue types can result in errors in $\delta^{13}C$ isotope values if not removed from the tissue prior to measurement (Post *et al.*, 2007). Therefore, all source and consumer samples underwent lipid correction of three 8 ml washes (or until the supernatant was clear) of 2:1 chloroform:methanol solvent according methodology developed by Bligh and Dyer (1959). Samples were again dried in the oven at 60˚C for 48 h to remove any remaining solvent. Aliquots of lipid extracted tissue of 400-600µg were weighed into tin capsules for stable isotope analysis.

Stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of all samples were measured at the Stable Isotope Core Laboratory of Washington State University using an elemental analyser (ECS 4010, Costech Analytical, Valencia, CA) connected to a continuous flow isotope ratio mass spectrometer (Delta PlusXP, Thermofinnigan, Bremen) and expressed as parts per thousand (‰) (further details can be found in the Supplementary Methods A2.1 in appendix 1).

2.2.4. Data analysis

The package SIMMR - Stable Isotopes Mixing Models in R (Parnell, 2016) was used to estimate the likely contribution of each putative food source to the diet of *Nephrops* by solving mixing equations for stable isotopic data within a Bayesian framework. SIMMR model outputs are posterior probability distributions representing the likelihood of a specific source being part of the diet of the consumer, with their respective credible intervals. SIMMR was run based on the following input data: $\delta^{13}C$ and $\delta^{15}N$ isotope values of consumers, mean $\delta^{13}C$ and $\delta^{15}N$

isotope values of sources i.e. putative prey groups and their standard deviations and estimates for ^{13}C and ^{15}N trophic enrichment factors (means and standard deviations – see below).

For the initial analysis, to show the importance of POM_{susp} in the diet and to ensure that all dietary sources were captured in the analysis, sources were divided into seven taxa/groupings: (i) Crustaceans; (ii) Filter feeders; (iii) Fish; (iv) Phytoplankton; (v) Polychaeta; (vi) POM_{susp} and (vii) Zooplankton. Meanwhile, consumers were grouped in all possible combinations of size (small, medium, large), sex (male and female) for long / short-term storage tissues (respectively, muscle and hepatopancreas), providing 12 different combinations overall. Comparison of diet between these consumer groups formed the basis of further hypothesis testing, i.e. statistical comparisons of 'active feeding' versus 'suspension feeding', as described in 'Statistical design', below. The SIMMR model was run twice based on isotope values (for both consumers and food sources) collected in each of the first and second sampling days. Next, four experimental time *'Periods'* were defined based on the combination of the two sampling dates and two different tissues representing a long (muscle) or short (hepatopancreas) residence times (rt) (Table 2.1). Residence time for muscle tissue was 81.1 days, obtained from isotopic incorporation rates and discrimination factors in *Neogonodactylus bredini* (mantis shrimp) (deVries *et al.*, 2015), while rt for hepatopancreas was estimated as 19.3 days. This was calculated from the ^{13}C half-life for hepatopancreas tissues in *Callinectes sapidus* (Vedral, 2012) (further details of these calculations can be found in the Supplementary Methods A2.1 in appendix 1).

Trophic enrichment (or 'fractionation') factors (TEFs) of 3.0 ± 0.6 ‰ for $\delta^{13}C$ and 0.9 ± 0.3 ‰ for $\delta^{15}N$ were chosen, based on estimates from mantis shrimp muscle (deVries *et al.*, 2015), which is the best taxon-specific information available. These values contrast with widely-used values from previous meta-analysis (McCutchan *et al.*, 2003) that present averages from 61 different species of aquatic and terrestrial vertebrates and invertebrates in a variety of taxa:

arthropods, molluscs, nematodes, birds, fish and mammals (for information, values in McCutchan *et al.* (2003) were 0.5 ± 0.13 ‰ for $\delta^{13}C$ and 2.3 ± 0.18 ‰ for $\delta^{15}N$). Nevertheless, we chose the mantis shrimp values (deVries *et al.*, 2015): firstly, because McCutchan *et al.* (2003) TEF values showed to be inadequate, since when using them, $\delta^{13}C$ and $\delta^{15}N$ isotope values of consumers laid outside the mixing polygon; secondly, on the basis that these fractionation values were calculated from a decapod crustacean: taxonomic relatedness is important due to evidence that TEFs are taxon-specific due to shared physiological processes at taxon level (Vanderklift and Ponsard, 2003; Caut *et al.*, 2009; Suring and Wing, 2009; del Rio and Carleton, 2012; Remy *et al.*, 2017); finally, the values in deVries *et al.* (2015) represented lipid-corrected stable isotope ratios for consumers and prey, as also used in our study, and were from a diet shift-controlled laboratory experiment.

2.2.5. Statistical design

Each group of consumers subjected to hypothesis testing included 10 replicate consumer samples (n = 10). This sample size seems adequate in bootstrapped simulations, which have shown an absence of large biases in statistical inference of stable isotope data with >8 replicate consumer samples (Pearson and Grove, 2013). For hypothesis testing, sources were combined by the function '*combine_sources'* of SIMMR package into 'active feeding' (i.e. filter feeders, polychaete, crustaceans and fish) and 'suspension feeding' (i.e. POM_{susp}, phytoplankton and zooplankton). In order to test our hypotheses, posterior distributions of 'suspension feeding' by consumers were compared in several ways. Size-related differences in consumers were examined across all 8 possible 'Sex' x 'Period' combinations (i.e. all combinations of males and females in 4 time periods). Sex-related differences in consumers were examined across 6 combinations of 3 'Size' groups in 2 periods, *'Spring long'* and *'Summer long'*. These periods represent an equivalent number of feeding days but with the key difference that *'Spring long'* included part of the period where females were brooding embryos in Clew Bay, i.e. up until

~10th April (Power *et al.*, 2019), whereas *'Summer long'* was a non-brooding period. Any dietary differences associated with female brooding could be judged against males using this comparison. Please note that, as they had just completed their reproductive cycle and had spawned, none of the females sampled actually contained embryo masses, however we could assume that 84-92% of our sample (n= 60) of females had bred, based on previous work (de Figueiredo and Thomas, 1967; Thomas, 1964; Thomas and Figueiredo, 1965; Farmer, 1974c). The suspension feeding contribution was compared across each of the above groups using the function *'compare_groups'* from the SIMMR package. This function gives the probability p_{BIC} of 'any diet source's proportion in one treatment being greater than the proportion of the same source in another treatment' with $p_{BIC} > 0.95$ considered to indicate significant differences (Masson, 2011).

The trophic position of *Nephrops* was determined based on the isotope values of consumers and prey according to a modified version of the following equation (Vander Zanden and Fetzer, 2007):

$$TP = \lambda + (\delta^{15}N_c - \delta^{15}N_{base})/\Delta_n \qquad \text{(eq. 2.1)}$$

Where $\delta^{15}N_c$ is the isotopic signature of the consumer *Nephrops*, N_{base} is that of the food base (herein filter feeders), λ is the trophic position of the base (λ = 2 for primary consumers) and Δ_n is an estimate of the average increase in $\Delta^{15}N$ per trophic position/level, herein set at 3.4 ‰ based on estimates for aquatic food webs (Post, 2002; Vander Zanden and Fetzer, 2007). However, because the TEF for δ^{15}N, is significantly lower for decapods (deVries *et al.*, 2015) than for many other taxa (Post, 2002; McCutchan *et al.*, 2003; Vander Zanden and Fetzer, 2007), we modified the eq. 2.1 to incorporate this and prevent an erroneous underestimation of *Nephrops'* trophic position, as follows:

$$TP = 1 + (\lambda + ((\delta^{15}N_c - 0.9) - \delta^{15}N_{base})/\Delta_n) \qquad \text{(eq. 2.2)}$$

Where 0.9 is the TEF for $\delta^{15}N$ of mantis shrimp (deVries *et al.*, 2015) corresponding to the average increase in $\Delta^{15}N$ per trophic position/level; this was subtracted from the isotope values of the consumers in eq. 2.1 to facilitate a more accurate trophic position calculation for a decapod, as is the case in the present study. Because this manipulation of the equation underestimates the trophic position in one level, a correction was required by adding one trophic position / level at the end of the calculation, as seen in eq. 2.2.

For estimating *Nephrops*' overall trophic position, the isotope values in the tissues (muscle and hepatopancreas together) of all consumers (n = 120) and filter feeder sources (baseline) sampled in both sampling days were used. The overall trophic position, as well as the ones concerning the experimental time periods: *'Spring long'*, *'Spring short'*, *'Summer long'* and *'Summer short'* were estimated according to a Bayesian approach by the package tRophicPosition (Quezada-Romegialli *et al.*, 2016) for R environment. The mean value and standard deviation (μ= 3.27 and sd = 0.42, respectively) of previous trophic position estimates (Loc'h and Hily, 2005; Hill, 2007; Watts, 2012) were used to build a normal distribution which was used as prior distribution in the estimation of the trophic position. Trophic position estimates were compared by the function *'compareTwoDistributions'* from the package tRophicPosition (Quezada-Romegialli *et al.*, 2016) with $p_{BIC} > 0.95$ considered to indicate significant differences between trophic positions according to the Bayesian paradigm (see above).

2.3. Results

2.3.1. Importance of suspended particulate organic matter to *Nephrops'* diet

Consumer isotopic values fell within the range of food source isotopic values for all four time periods (Figure 2.1), indicating that all major *Nephrops*' dietary sources were included in the analysis and no important dietary sources were missing. This arrangement between consumer and source values is a precondition for the SIMMR Bayesian mixing model to work adequately

(Phillips *et al.*, 2014). The SIMMR model output indicated that POM_{susp} and fish were the main food sources for all consumer groups and time periods in the analysis. The estimated means of POM_{susp} contribution to the diet ranged between 12.0 - 47.4% but was generally high, >20% (Supplementary Table A2.3 in appendix 1). Meanwhile, apart from fish, contributions from the other sources (phyto- and zooplankton, filter feeders, polychaetes and crustaceans) were much lower, with means ranging from 2.3-15.6% (Figure 2.2). The estimated means for fish contribution to the diet ranged from 18.2 - 60.9%. Therefore, the main question about the importance of POM_{susp} in *Nephrops*' diet was accepted to be the case in this study. After combining sources, the estimated means of 'active feeding' (i.e. filter feeders, polychaete, crustaceans and fish) ranged from 42.5-76.2%, while that of 'suspension feeding' (i.e. POM_{susp}, phytoplankton and zooplankton) ranged from 26.5-57.5%. More details on average contributions from all food sources and probability distributions of 'active feeding' and 'suspension feeding' to *Nephrops*' diet can be seen respectively in Supplementary Table A2.3 and Supplementary Fig. A2.1 in appendix 1.

2.3.2. Size-related differences in *Nephrops*' diet

There were some significant *Nephrops* size-related differences in suspension feeding (i.e. POM_{susp}, phytoplankton and zooplankton). The results showed that suspension feeding took place significantly more in small or medium sized individuals than large ones. Surprisingly, this occurred more frequently in male comparisons than in females. Suspension feeding was significantly higher in the small and medium sized males compared to larger males in the early time period, i.e. *'Spring long'*: 8th March – 29th May (Figure 2.3). In the late time period, i.e. *'Summer long'* (4th May - 25th July), it was significantly higher only in the small males compared to large males (Figure 2.3). For females, the small size class was significantly more likely to suspension feed than larger ones in the *'Spring short'* period (10th - 29th May) (Figure 2.3). No significant differences could be detected between the size groups in either sex in the

'Summer short' time period (6th - 25th July) (Figure 2.3). significant size-related difference in suspension feeding in the diet i.e. $p_{BIC} > 0.95$ (provided by the SIMMR package according the Bayesian paradigm).

2.3.3. Sex-related differences *in Nephrops*' diet

The comparison of suspension feeding between males and females produced no significant results across time periods including: 8th March – 29th May (*'Spring long'*), which includes part of the long breeding season of females, and 4th May – 25th July (*'Summer long'*) when both sexes are non-burrow dwelling and capable of feeding outside of burrows (Figure 2.4). The contribution of active feeding and suspension feeding to the diet of males and females was also equivalent, with imperceptible differences observed between the sexes (Supplementary Fig. A2.1 in Appendix 1). Thus, the hypothesis of differences between male and female's diet, related to the reproductive cycle of females is not upheld at Clew Bay.

2.3.4. Trophic position of *Nephrops norvegicus*

The overall trophic position of *N. norvegicus* in Clew Bay, based on isotopic signatures in muscle and hepatopancreas (considered together) was estimated to be 3.50, which represents the mean of the posterior distribution over all experimental time periods. The trophic position across different periods varied from 3.26 (*'Spring short'*) to 3.72 ('*Summer long'*). See Table 2.2 and Figure 2.5 respectively for trophic position estimates and 95% Bayesian credible intervals for those estimates. The trophic position estimates for the time periods *'Spring short'* and *'Summer long'* were significantly different between them and from the other estimates (*'Spring long'*, *'Summer long'*, *'Summer short'* and *'Overall'*) with $p_{BIC} > 0.95$ (see non-overlapping 95% credible intervals in Figure 2.5).

2.4. Discussion

The primary aim of this research was to investigate feeding on suspended particulate organic matter (POM_{susp}) in wild *Nephrops* as this may be a mechanism of avoiding seasonal starvation.

Before looking at its contribution to the diet, we could establish that we had captured all the important dietary sources by the arrangement of isotope values in the $\delta^{13}C$ / $\delta^{15}N$ bi-plots (Figure 2.1). The position of consumer tissues within the polygon of sampled food sources for each of the four periods indicated that no important food source(s) were missing in the analysis. It is important to emphasize that Bayesian SIA models are extremely sensitive to variations in the values of TEFs (Bond and Diamond, 2011). Indeed, if the commonly used TEF values for marine consumers were used in the analysis: 0.5 ± 0.13 ‰ for $\delta^{13}C$ and 2.3 ± 0.18 ‰ for $\delta^{15}N$ (McCutchan *et al.*, 2003) instead of the taxon-specific one used herein: 3.0 ± 0.6 ‰ for $\delta^{13}C$ and 0.9 ± 0.3 ‰ for $\delta^{15}N$ (deVries *et al.*, 2015), the isotope values of consumers would lay outside the polygon of sampled food sources for each of the four periods and thus the analysis would be useless. Trophic enrichment factors are undoubtedly a critical point in SIA, since they might be influenced by many aspects e.g. taxa, diet, tissue type, environment and lipid extraction treatments (Caut *et al.*, 2009). Perhaps, further investigations might confirm deVries et al. (2015)' TEF values as appropriate for SIA of *Nephrops*' diet or even determine plausible values for them, considering all aspects above-mentioned or any other that might influence those values.

At times, almost half (47%) of the diet of *Nephrops* was made up of POM_{susp}. These lobsters did show variety in their diet, however, and another important item in the diet was fish, while plankton and invertebrate sources came far below these items in dietary importance. Reliance on POM_{susp} and fish, rather than on invertebrates, appears initially surprising considering predominance of invertebrates in stomach content analysis (Parslow-Williams *et al.*, 2002; Bell *et al.*, 2013). However, many crustaceans are predatory on fish, which is apparently independent of their size (Hickman *et al.*, 2001). Capture of flatfish, for example, may present little difficulty to *Nephrops*, whose diet may also be subsidised from discards arising from inshore fishing activity in Clew Bay. The high level of feeding on particulate matter was more

surprising, however another burrowing decapod crustacean, *Neotrypaea californiensis*, has recently been shown to be primarily reliant on POM_{susp} as a food source (Bosley *et al.*, 2017). Due to their burrowing lifestyle and long breeding season in females, much effort has gone into investigating seasonal starvation in *Nephrops* (Mente *et al.*, 2011; Rotllant *et al.*, 2014; Watts *et al.*, 2014, 2016). The ability to feed on particulate food sources would help to counteract starvation brought about by these lifestyle restrictions. When *Nephrops* individuals were maintained in unfiltered seawater in an aquarium, they showed an intermediate nutritional status between control animals with no access to food and those from the wild, which was suggestive of suspension feeding, at least *in-extremis* with no other food available (Loo *et al.*, 1993). The present results add to this by demonstrating the importance of suspension feeding to the diet of wild individuals, showing that they utilised this food source at a significant level. Previous work has theorised that 65-68% of daily energy intake was available for growth from suspension feeding at sufficient particulate densities (Parslow-Williams *et al.*, 2002). Although our study does not address energy intake directly, our estimates of suspension feeding in the diet often reached 50% (particularly in short-term tissues). This likely represents a considerable amount of suspension feeding-derived energy available for growth (Supplementary Table A2.3 in appendix 1).

In fact, it has long been acknowledged that POM_{susp} is an important seasonal source of food for benthic organisms in winter (Riley, 1971; Darnaude, 2005). Not all suspended food particles were equally important, however, for example phyto- and zooplankton were far less important than POM_{susp} in *Nephrops* (Figure 2.2; Supplementary Table A2.3 in appendix 1). Sediment organic matter (SOM) could be another important food source for benthic organisms like *Nephrops*. A practical difficulty is distinguishing SOM from POM_{susp} because the latter eventually falls to the seafloor and therefore forms one component of the SOM. Although we cannot discount the possibility that, as well or instead of feeding on POM_{susp}, *Nephrops* also

picks POM up off the sediment while deposit feeding on a mix of SOM/POM, we do not have evidence to support this idea. POM_{susp} and SOM can show distinct isotopic signatures (Ziolkowska *et al.*, 2018). For example, compared with POM_{susp}, SOM was shown to be enriched in $\delta^{13}C$ and depleted in $\delta^{15}N$ (Ziolkowska *et al.*, 2018). Had SOM with this (Ziolkowska *et al.*, 2018) profile been substituted for POM_{susp} in the present study, *Nephrops* samples would have fallen outside the isotopic polygon. Also, the $\delta^{13}C$ / $\delta^{15}N$ bi-plot in our study showed no missing dietary items, which might have been expected had SOM been important in the diet. Other studies have also shown distinct POM and SOM signatures (Hill, 2007; Carrasco and Perissinotto, 2010). Future studies combining fatty acid analysis and SIA may further disentangle the various sources of organic particulates and their relative importance, including sources found inside lobster burrows, e.g. Bosley et al. (2017). Furthermore, next generation sequencing (NGS) such as DNA metabarcoding that allows the characterization of many consumed species simultaneously (Pompanon *et al.*, 2012) may be an important tool to improve feeding ecology studies concerning *Nephrops* or other decapod species.

The hypothesis suggesting a size-related difference in *Nephrops* diet was accepted for several comparisons. Suspension feeding was higher in smaller compared to larger size classes for males in particular, e.g. during *'Spring long'*: 8th March – 29th May (small and medium males compared to large males) and '*Summer long*': 4th May – 25th July (small males compared to large males). Males may suffer more competition for active food items than females (Merder *et al.*, 2019) which may force smaller individuals to rely on particulate food sources. The same size-related difference, i.e. a higher proportion of suspended food in the diet of small females compared with larger ones, was borne out in only one time period: the *'Spring short'* feeding on 10th – 29th May. This was not seen for the equivalent tissue later in the season '*Summer short*' on 6th – 25th July. Interestingly, the size-related differences we observed did not appear

to be related to a limitation on predation capacity in smaller lobsters. Indeed, at times, the contribution to the diet by fish was even higher in smaller individuals than in the larger ones, e.g. with mean values of 48.1% versus 44.0 % and 44.7% versus 28.1 % for small versus large males in two of the four periods analysed (Figure 2.2, Supplementary Table A2.3 in appendix 1). However, without further research it is difficult to interpret the reason for this, for example, it is possible that smaller individuals may feed on fisheries discard or on larger individual's leftover prey.

Although the isotopic signal from long and short-term storage tissues varied substantially, there was no difference in 'Spring' and 'Summer' diets when similar storage tissues were compared (Supplementary Fig. A2.1 in appendix 1). The difference between long and short-term storage tissues arises because these represent different time intervals, 19 and 81 days respectively. Active feeding was higher in long-term storage tissues, whereas suspension feeding was increased in short-term tissues (Supplementary Fig. A2.1 in appendix 1). Without further experiments, the reasons for this are unclear, however.

The hypothesis related to sex-specific diets was rejected. Males and females were remarkably similar in diet, even in the Spring period (8th March – 29th May), even though the tissues sampled from this time represented a period when females were mostly brooding. Larval release at Clew Bay begins around the second week of April (Power *et al.*, 2019), until which point, the females stay inside burrows to brood their developing embryos. Our results demonstrate that this period of burrow dwelling does not prevent females from accessing the same food items as males. It has been suggested that feeding by females during the breeding season may simply take place closer to the burrow mouth (Katoh *et al.*, 2013; Watts *et al.*, 2016) or females may bury food within or adjacent to burrows (Watts, 2012). Although the sexes have similar diets, as shown in the present study, the overall opportunity for feeding may be reduced in females. However, starvation and sex-specific reduction in nutritional status has

been previously examined and found to be absent (Watts *et al.*, 2016), with corroborating evidence from biochemical markers that suggest good nutritional condition throughout the year in females (Rotllant *et al.*, 2014). Although Clew Bay is a particularly shallow site, the same major food groups (i.e. plankton and particulates, macroinvertebrates and fish) are available in deeper habitats (Chapman and Rice, 1971; ; Gual-Frau and Gallardo-Cabello, 1988; Cristo and Cartes, 1998; Parslow-Williams *et al.*, 2002; Bell *et al.*, 2013; Watts *et al.*, 2016). *Nephrops* diurnal emergence does vary with depth (Lauria *et al.*, 2015) but we can think of no plausible reason for this to interact with the availability of POM_{susp} or other food groups. Therefore, we believe the results are transferrable to other *Nephrops* populations, although other locations may have slightly different groups of macroinvertebrates (echinoderms, in particular, were not abundant in the sediments at Clew Bay).

Based on isotopic signatures in the present study, 'Overall' trophic level was calculated to be 3.50 i.e. approximately in the middle point of the range of previous estimates for *Nephrops*: 2.60 – 4.32 (Loc'h and Hily, 2005; Hill, 2007; Watts, 2012) that were also derived using SIA. Trophic position at *'Spring short'* was significantly lower than the estimates at other time periods (*'Overall'*, *'Spring long'*, *'Summer short'* and *'Summer long'*), while an inverse trend was observed for the trophic position at *' Summer long'*, which was significantly higher than the ones at the other time periods (*'Overall'*, *'Spring short'*, *'Spring long'* and *'Summer short'*). This output is consistent with a higher and lower consumption of fish (48-61%) and POM_{susp} (12-23%) in *'Summer long'*, as well as with an inverse trend in *'Spring short'*: fish and POM_{susp} consumptions respectively around 18-25% and 32-47% (see Figure 2.2 and Table A2.3 in appendix). It is important to emphasize that SIA can reveal lower trophic status in consumers compared with stomach contents analysis, because the latter can underestimate soft-bodied prey (Wieczorek *et al.*, 2018). In the case of *Nephrops*, it would be almost impossible to detect the fact that up to half of the diet derived from POM_{susp} in stomach contents. Such

disproportionate measurement of prey items acts to artificially inflate trophic position based on stomach contents alone.

As the present study shows, smaller and medium-sized males fed on significantly higher suspended food than larger ones, therefore the potential to suspension feed may be an important mechanism for avoiding aggressive encounters over food between males. This is potentially important because the growth (and hence biomass) of male individuals is strongly density-dependent at Clew Bay (Merder *et al.*, 2019): - densities at Clew Bay vary between 0 and 15 individuals per pot fished (Power *et al.*, 2019). Body size also varies across fishing grounds - smaller *Nephrops* are found at FUs with higher stock densities, most likely as a result of reduced growth potential due to intraspecific competition (Johnson *et al.*, 2013; Merder *et al.*, 2019). We suggest that feeding on POM is an important lifestyle adaptation in both males (counteracting competitive interactions) and females (counteracting burrow-dwelling) but that *Nephrops* diet is remarkably similar in the sexes. The knowledge that fish is also an important component of the diet in all groups examined at Clew Bay means that, in theory, reduced subsidy from fisheries discards to scavengers like *Nephrops* under the EU Landings Obligation could affect feeding opportunities for this species in the future.

2.5. Tables

Table 2.1. Time periods sampled based on retention time of carbon and nitrogen stable isotopes in short and long-term storage tissues in *Nephrops* consumers collected on 2 sampling days in 2014 (Supplementary Methods A2.1 in appendix 1 provide more details about residence time calculations).

Period name	Sampling day	Tissue	Residence time	Period
Spring long	29th May	Muscle	81 days	8th March – 29th May
Spring short	29th May	Hepatopancreas	19 days	10th – 29th May
Summer long	25th July	Muscle	81 days	4th May – 25th July
Summer short	25th July	Hepatopancreas	19 days	6th – 25th July

Table 2.2. Trophic position of *Nephrops* in Clew Bay (2014), based on $\delta^{13}C$ and $\delta^{15}N$ values from different time periods represented by long-term storage tissues (muscle: *'Spring long'* and *'Summer long'*) and short-term storage tissue (hepatopancreas: *'Spring short'* and *'Summer short'*). See Table 2.1 for relevant time periods.

Period	Trophic Position
Spring long	3.53
Spring short	3.26
Summer long	3.72
Summer short	3.51
Overall	3.50

2.6. Figures

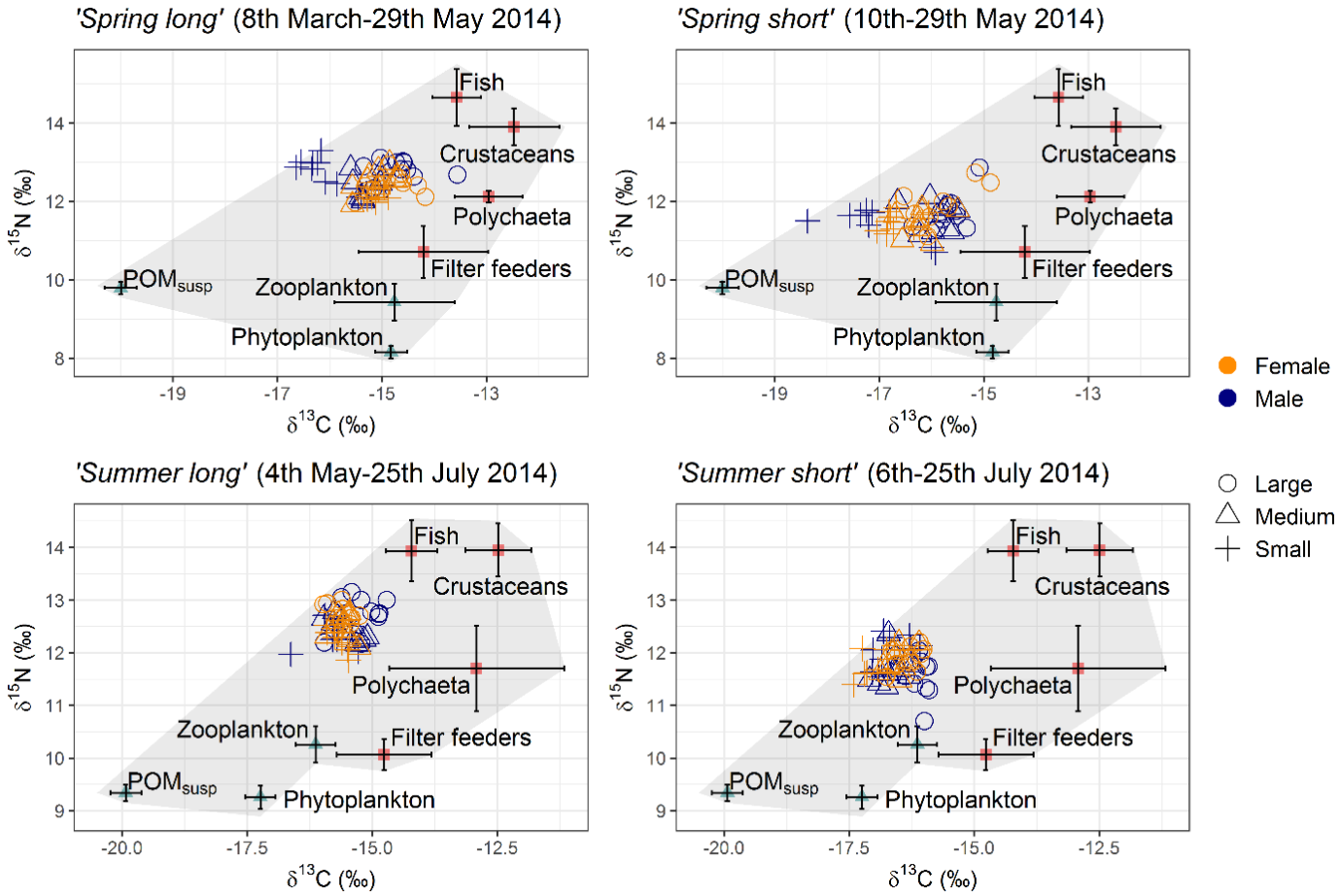

Figure 2.1. Stable isotope bi-plot of $\delta^{13}C$ and $\delta^{15}N$ values of consumers and food sources. The figure provides the isotope values of *Nephrops* individuals within a polygon representing their putative prey in different time periods at Clew Bay, Ireland, 2014.

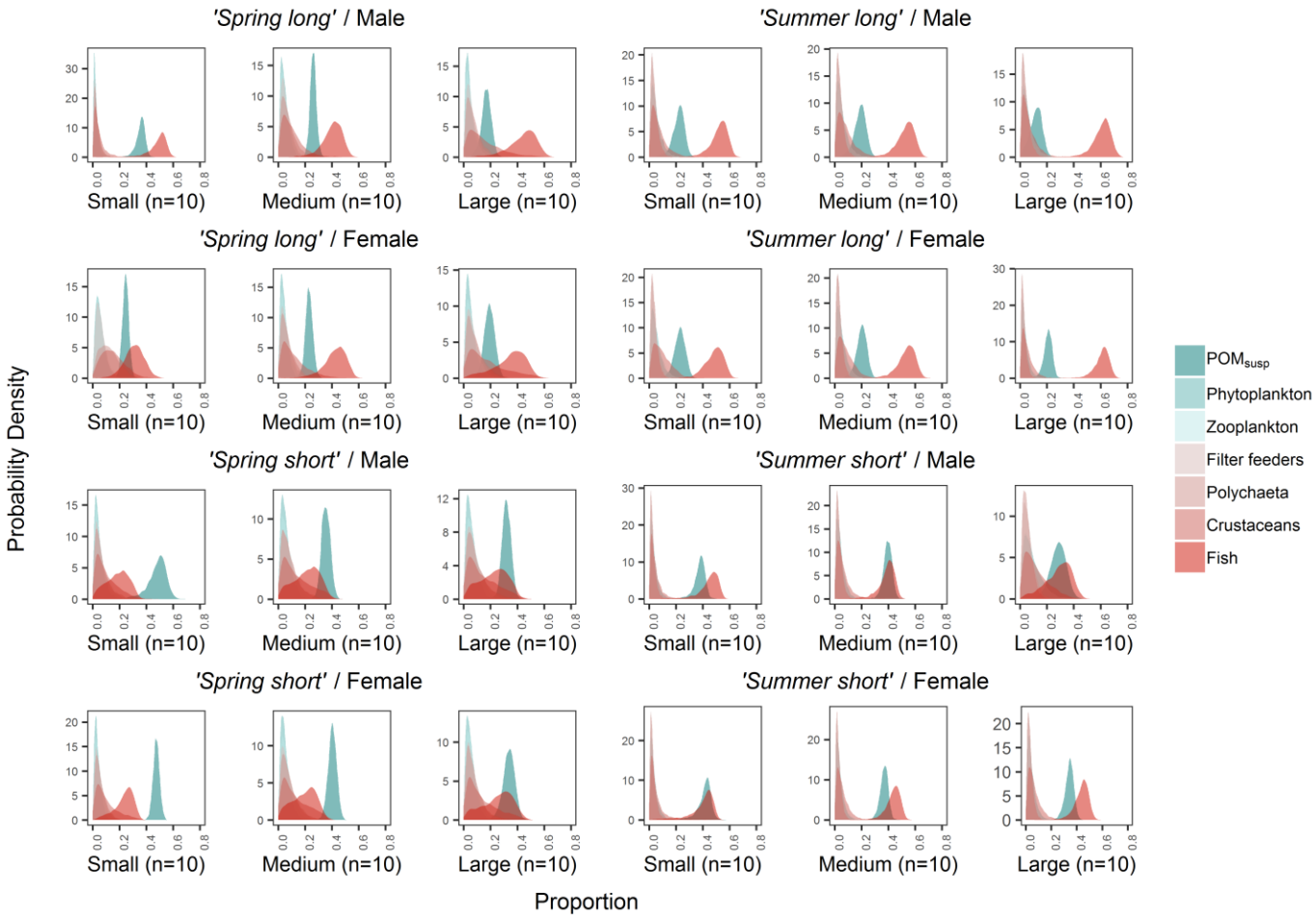

Figure 2.2. Contributions of putative sources to *Nephrops*' diet. The figure shows posterior probability distributions of the contributions of all putative sources to *Nephrops*' diet in different periods during Spring and Summer, 2014: *'Spring long'* (8th March–29th May), *'Spring short'* (10th–29th May), *'Summer long'* (4th May–25th July) and *'Summer short'* (6th–25th July).

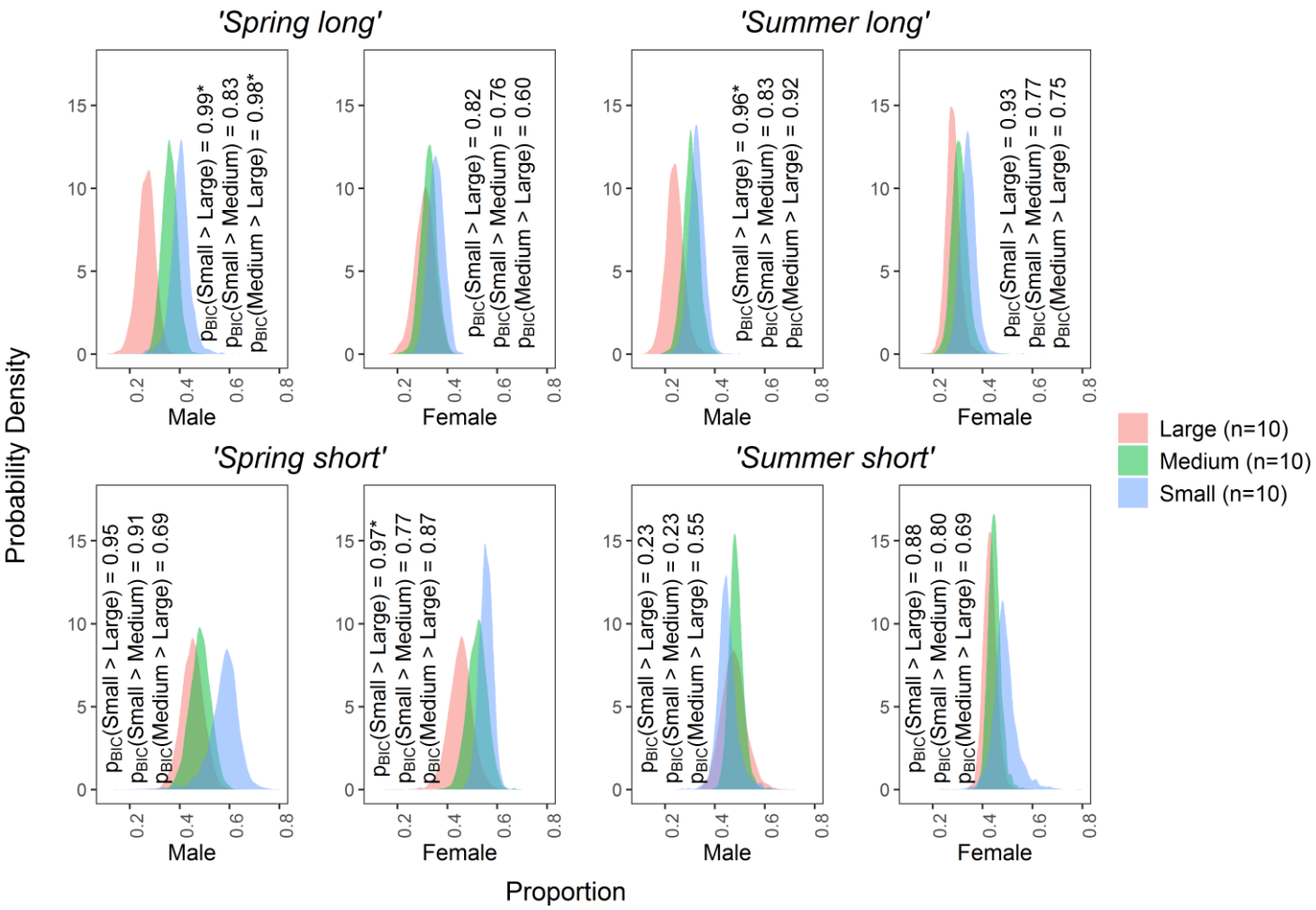

Figure 2.3. Size-related differences in *Nephrops*' diet from suspension feeding. The figure shows posterior probability distributions comparing contribution to the diet of suspension feeding (i.e. phytoplankton + zooplankton + POM_{susp}) in *Nephrops* of different sizes during Spring and Summer 2014: *'Spring long'* (8th March–29th May), *'Spring short'* (10th–29th May), *'Summer long'* (4th May–25th July) and *'Summer short'* (6th–25th July). An asterix indicates a significant size-related difference in suspension feeding in the diet i.e. p_{BIC} > 0.95 (provided by the SIMMR package according the Bayesian paradigm).

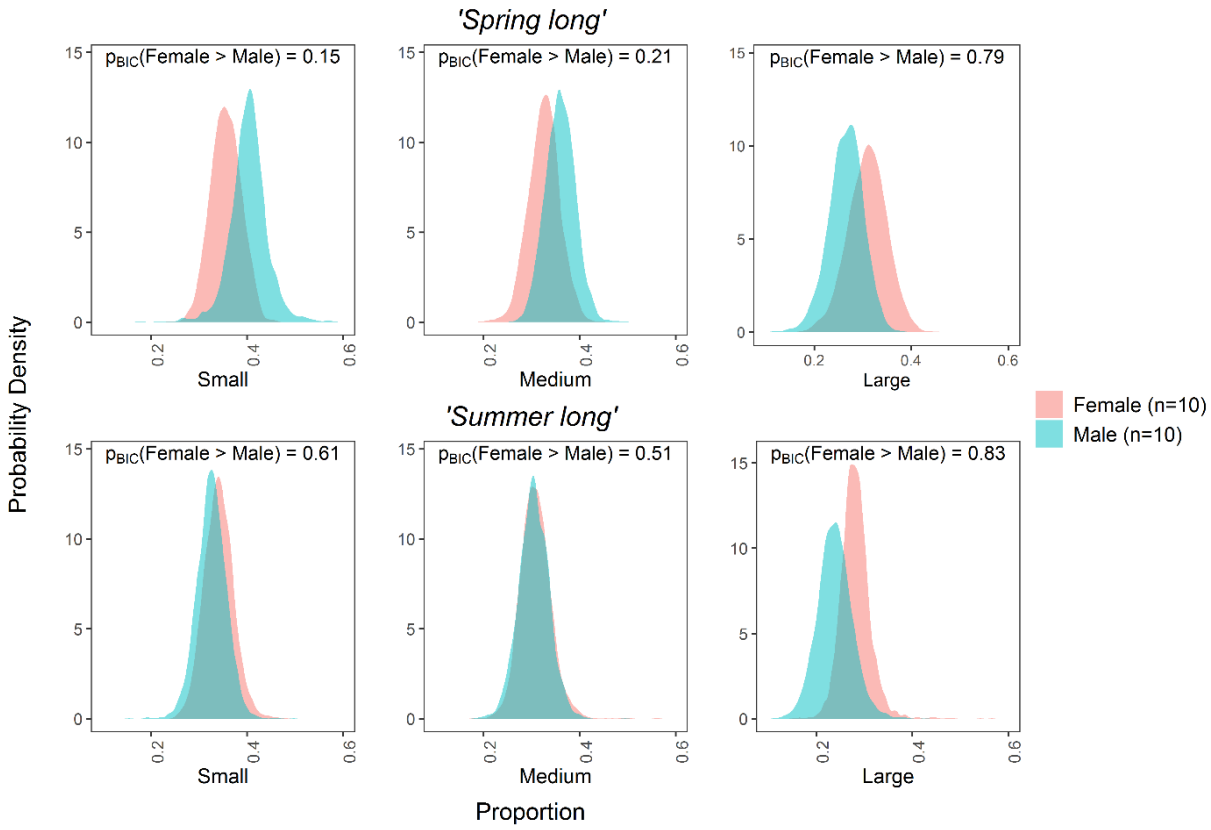

Figure 2.4. Sex-related differences in *Nephrops*' diet from suspension feeding. The figure shows posterior probability distributions comparing contribution to the diet of suspension feeding (i.e. phytoplankton + zooplankton + POM_{susp}) in *Nephrops* of different sexes, including periods when females were burrow-dwelling i.e. 8th March–29th May 2014 (*'Spring long'*) and non-burrow dwelling periods 4th May–25th July 2014 (*'Summer long'*). An asterix denotes a significant sex-related difference in suspension feeding in the diet i.e. $p_{BIC} > 0.95$ (provided by the SIMMR package according the Bayesian paradigm), however no such differences were observed.

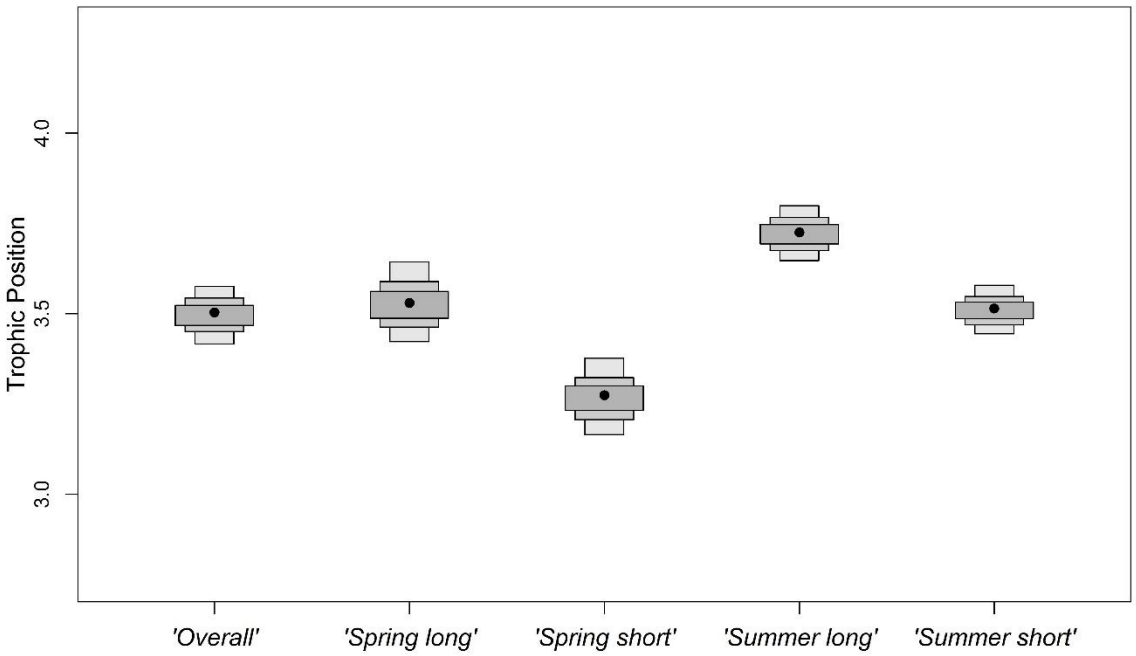

Figure 2.5. Trophic position estimates for different time periods: 8th March–29th May 2014 (*'Spring long'*), *'Spring short'* (10th–29th May), 4th May–25th July 2014 (*'Summer long'*) and *'Summer short'* (6th–25th July). *'Overall'* refers to the trophic position estimate considering isotope values of those different time periods grouped together.

Chapter 3

Tools to examine *Nephrops*' maturity

3. Theoretical size at the onset of maturity and its density-dependent variability as an option in crustacean fisheries management

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Author contributions: A.M.P. and C.A.S.S. obtained the funding, designed the study, carried out the statistical analysis, wrote the manuscript, prepared the figures and reviewed the manuscript; C.L. provided the dataset for TSOM estimation and reviewed the manuscript.

Abstract

Theoretical size at onset of maturity (TSOM) for female *Nephrops* (Norway lobster) was estimated by a new methodology based on probability distributions of mature individuals built on physiological maturity measures (size-dependent gonad staging). Onset of maturity using TSOM varied from 18.4-33.7 mm carapace length for the Irish functional management units (FUs). These estimates showed a significant negative linear relationship (R^2 = 0.60) with population density at all FUs / years, and a significant positive linear relationship with average size in females (both mature and immature, R^2 = 0.84). L_{50} was linked to the new TSOM metric by a significant positive linear relationship (R^2 = 0.40). This set of linear relationships ultimately allowed TSOM and L_{50} to be estimated without a requirement for maturity stages to be distinguished. As well as contributing to stock assessment and management of *Nephrops* (e.g. in data-limited FUs) and its potential for calibration of more routinely-used estimates, TSOM might be applied in new species and meta-analyses where size of maturity data are scarce. This new metric also better-defines the maturity process since, taken together, TSOM,

L_{50} and SBF represent sequential maturity events: i) onset of maturity, ii) 50% mature (from gonad staging) and iii) berried females.

3.1. Introduction

The beginning of sexual maturity presents great variability among species, populations of the same species or even among individuals of the same population (Fonteles-Filho, 1989). The range of size classes at which individuals in a population reach sexual maturity is used to estimate a proxy that is used to indicate the size at which a population or stock reach the sexual maturity, i.e. size of maturity (SOM) (Fonteles-Filho, 1989). SOM is important for stock assessment and management of valuable fisheries resources as this directly impacts various management activities and practices, from the estimation of the spawning stock potential to adoption of a minimum landing size e.g. Hilborn and Walters (1992). Traditionally, size and age of maturity have been considered static for the purposes of stock assessment, however contemporary research indicates potential spatial and temporal variation in SOM related to fishing pressure and population density (Lowerre-Barbieri *et al.*, 2011; Queirós *et al.*, 2013; Haig *et al.*, 2016; Waiho *et al.*, 2017). As a consequence, periodic estimation of these life-history parameters is recommended. This is all the more important since density-dependent size structure has been shown in crustacean species such as Norway lobster, southern rock lobster, Oregon shore crab and sharp-nosed crab (Bailey and Chapman, 1983; Hines, 1989; Briggs, 1995; McGarvey *et al.*, 1999; Tuck *et al.*, 2000; Johnson *et al.*, 2013), as well as in different fish species e.g. bicolor damselfish, perch, flatfish and haddock (Lorenzen and Enberg, 2001; Hixon *et al.*, 2012). For some crustacean species, this density-dependent size structure also seems to scale with SOM (Hines, 1989; Briggs, 1995; Tuck *et al.*, 2000; Queirós *et al.*, 2013). SOM may also scale with fishing pressure, e.g. in the rock lobster, although the genetic and ecological drivers were difficult to separate (Pollock, 1995). Methods to estimate the SOM quickly, reliably and routinely are therefore required.

Sexual maturity is a gradual process that includes gonad development, gametes maturation and liberation of spermatozoids, ova or fry, to the environment (Fonteles-Filho, 1989). It is associated with alterations in both external morphology and internal physiology, on which bases different types of maturity can be defined; physiological, morphometric and functional (Waiho *et al.*, 2017). Physiological maturity is defined as the capacity of individuals to produce gametes (Waiho *et al.*, 2017). In crustaceans, this is directly observed via primary sexual characteristics: gonadal development and colouration in females and presence of spermatophores in the vasa deferentia in males (McQuaid *et al.*, 2006; Queirós *et al.*, 2013; Haig *et al.*, 2016; Waiho *et al.*, 2017). Morphometric maturity, on the other hand, is inferred based on allometry in the growth of certain body structures (i.e. secondary sexual characteristics) that may occur at the onset of sexual maturity (MacDiarmid and Saint-Marie, 2006). This allometry is theoretically related to physiological and biochemical changes during sexual maturation, including differential somatic growth, activities associated with mating behaviour and associated energy expenditure (Queirós *et al.*, 2013; Haig *et al.*, 2016). For example, alterations in the allometry of chelipeds / appendix masculina (males) or abdomen width (females) are essential structures related to antagonistic behaviour / courtship / copulation (males) or an enhanced capacity to accommodate eggs (females) (Farmer, 1974a; McQuaid *et al.*, 2006; Queirós *et al.*, 2013; Haig *et al.*, 2016; Waiho *et al.*, 2017). It is important to stress that morphometrics remain an indirect measure of maturity that are potentially complicated by slight differences in phenotypes from place to place (the latter may or may not be associated with maturation). Finally, functional maturity is defined as the ability of individuals to mate successfully and to produce offspring (Queirós *et al.*, 2013; Haig *et al.*, 2016; Waiho *et al.*, 2017). This measure requires individuals to be physiologically and morphometrically mature (Evans *et al.*, 1995; Haig *et al.*, 2016; Waiho *et al.*, 2017).

In female crustaceans, maturity is generally estimated by direct methods i.e. observation of physiological characteristics (Farmer, 1974c; Jayakody, 1989; MacDiarmid, 1989; Briggs, 1995; Bianchini *et al.*, 1998; Tuck *et al.*, 2000; Mente *et al.*, 2009; Marković *et al.*, 2016; Peixoto *et al.*, 2018). However, there is a variety of physiological-based criteria to define size of maturity in female crustaceans, such as, the smallest female with ripe ovaries, carrying spermatophores or eggs, the size class most frequently carrying eggs, the mode of all females carrying eggs, and so on (Evans *et al.*, 1995; McQuaid *et al.*, 2006). Estimating the SOM then becomes a complicated issue since this will depend on the method employed in the estimation and without standardization, these estimates are not easily compared (Lowerre-Barbieri *et al.*, 2011; Haig *et al.*, 2016). Unfortunately, estimates based on these primary characters are prone to bias due to seasonality in the female reproductive cycle, which can impose sampling artefacts (Queirós *et al.*, 2013). For males, the issue is even more complicated, where the determination of physiological maturity requires observation of internal structures by expensive and time-consuming histological techniques (Farmer, 1974c; Rotllant *et al.*, 2012). Once the maturity metric is decided and data collected, estimates can be compared using the size class at which 50 % of the females are sexually mature (L_{50}) (Morizur, 1983). However, these estimates may still be under- or over-estimated due to the difficulty in sampling smaller and immature individuals, or due to misclassifications e.g. females with regenerating ovaries that can be improperly classified as immature (Fonteles-Filho, 1989; Lowerre-Barbieri *et al.*, 2011).

Estimating SOM from morphometric data i.e. morphological maturity or 'MSOM' has been proposed as an alternative to physiological-based methods, since these are easier to obtain (at least for males), less prone to seasonal biases, and enable identification of maturity stanzas independent of gonad development (MacDiarmid and Saint-Marie, 2006; McQuaid *et al.*, 2006; Bell *et al.*, 2013; Queirós *et al.*, 2013). Indeed, this methodology has been widely used in crustaceans including edible crab (Haig *et al.*, 2016; Öndes *et al.*, 2017), Caribbean lobster

(Cusba and Paramo, 2017), Urugayan lobster (Severino-Rodrigues *et al.*, 2016); spiny lobster (Evans *et al.*, 1995), spotted spiny lobster (Evans *et al.*, 1995; Wynne, 2016), mangrove crab (Leme, 2005) and marbled fiddle crab (Hirose *et al.*, 2013). Morphological maturity is generally identified after investigating changes in allometric relationships, usually size of structure 'x' relative to carapace, identifying a statistically significant 'breakpoint' in that relationship, usually via partial regression. Still, Conan *et al.* (2001) suggest that bivariate analysis, on which identification of MSOM relies, may be inappropriate in crustaceans, due to inconspicuous growth stanzas in this taxon. Previous studies on morphological maturity in Norway lobsters estimated MSOM based on different body structures and geographical areas to be approximately 16-34.6 mm and 24-64.09 mm carapace length (CL) for females and males, respectively (Farmer, 1974a; Hillis, 1981; Mori *et al.*, 1996; Maynou and Sardà, 1997; Tuck *et al.*, 2000; McQuaid *et al.*, 2006; Queirós *et al.*, 2013). One drawback related to the MSOM estimation in this species was the great variability arising from use of different body structures, which prevented a reasonable choice of the most appropriate MSOM, as seen in Queirós *et al.* (2013).

To further complicate matters, morphometric and physiological maturities are not necessarily synchronized and represent different snapshots in the individual reproductive cycle that prevent absolute comparisons between them (Haig *et al.*, 2016; Waiho *et al.*, 2017). Lowerre-Barbieri *et al.* (2011) note the absence of methods that can definitively distinguish between immature and mature marine organisms, stating that approaches linked to other features of the maturation process, such as brain chemistry, endocrinology or aspects of the liver (due to its active role in vitellogenesis), might address this issue. However, improvements in analytical approaches such as probabilistic maturation reaction norms and statistical techniques could also be important tools to address this matter (Lowerre-Barbieri *et al.*, 2011).

The main aim of the present study was to propose a new methodology for estimation of the size at the onset of maturity called the theoretical size at the onset of maturity (TSOM). The proposed methodology is based on probability distributions of mature female carapace lengths that were built according to physiological maturity information provided from annual stock assessment exercises. Theoretical probability distributions of immature female carapace lengths were then created using information extracted from the probability distributions of the mature ones. The new method uses data routinely collected in stock assessment exercises by national responsible organizations. This fact simplifies the task of estimating the size at the onset of maturity and is less time-consuming than other approaches.

The particular case study for this new application is the Norway lobster *Nephrops norvegicus* (henceforth called *Nephrops*) which is the most valuable crustacean species exploited across the Europe Union with capture of 56,696 tonnes in 2017 (FAO, 2020). This species can be found on the continental shelf and slope across the northeast Atlantic, south to Portugal, as well as on grounds in the Mediterranean, Adriatic and Aegean seas, where it inhabits semi-isolated muddy grounds that are assessed by ICES as separate functional management units (FUs) (Johnson *et al.*, 2013). Due to occasional over-exploitation of some of these FUs (Anon., 2011; Lordan *et al.*, 2013), there is some concern about the sustainability of this species and employment of robust methods in its assessment and management are necessary.

Secondary objectives in the present study were to estimate the TSOM of female *Nephrops* at different Irish functional units, as well as to test the hypotheses of an inverse relationship between TSOM and population density (Sardà, 1995; Queirós *et al.*, 2013). To determine whether the new method is robust, TSOM was compared with existing measures including: smallest berried female (SBF), size at 50 % maturity (L_{50}, which represents the 'industrial standard' in routine use currently), before examining relationships between TSOM, average carapace lengths (immature and mature) and L_{50}.

3.2. Methodology

3.2.1. Theoretical size at the onset of maturity

3.2.1.1. Dataset

The dataset used for estimation of the TSOM in females was provided by the Marine Institute (Republic of Ireland). It consisted of annual time series (2001-2018) of *Nephrops* carapace length (CL) across Irish FUs (Table 3.1) with classification of physiological maturity stages (females) according to Mente *et al.* (2009). From this dataset, females were distinguished as either (i) immature or (ii) mature, where (i) corresponded to maturity stage 1 termed 'Female_Pale' in the dataset, and (ii) corresponded to maturity stages 2-6, i.e. maturing, sexually mature, and ovigerous females, respectively termed 'Female_Medium', 'Female_Dark' or 'Female_Eggs' in the dataset.

3.2.1.2. Probability distributions of mature females

Subsets of mature female CLs were obtained from annual samples of each FU in the time series (sample sizes are given in Table 3.1). Probability distributions of CLs were fitted for each subset by the R package 'fitdistrplus' (Delignette-Muller and Dutang, 2015). Different forms of probability distributions (normal, lognormal or gamma) were fitted depending on the FU / year (fitted distributions and respective goodness-of-fit plots are provided in Supplementary Figures A3.1-3.6). Females considered for probability distribution fitting purposes were the ones that had already started the process of maturation and were classified as maturity stages 2-6 as stated above. Critical points (CP_{mature} in Figure 3.1) were estimated by the function 'quantile()' of the 'fitdistrplus' package, such that the cumulative probability of any mature female with a carapace length (CL_{mature}) less than this critical value was $P(CL_{mature} < CP_{mature}) = 0.05$, i.e., CP_{mature} was defined as a cut-off for female CLs, below which is not common to find any mature individuals

3.2.1.3. Probability distributions of immature females

The original idea was to fit probability distributions of immature female CLs by the same methodology used for the mature ones. However, it was observed that probability distributions fitted using this procedure seemed to be biased, possibly because i) probability distributions of CLs from samples of immature individuals are expected to be right-skewed (as larger individuals are more likely to be sampled) and ii) populations follow the exponential distribution of survivorship governed by the "Type III" Deevey type curves, which leads to higher relative frequency of smaller and immature females only in the initial cohorts (Deevey, 1947; Ricker, 1975; Sparre and Venema, 1998). Also, field sampling methods may not adequately take smaller individuals into account because, as suggested by other studies, this category of individuals is largely restricted to burrows to avoid predation or antagonistic confrontations, and is rarely seen in the catches as a result (Santana *et al.*, 2020). Observe, for example, the plot of real frequency data of a sample of immature females in this study and the fitted normal probability density curve in Figure 3.2. The shaded area in grey under the curve refers to the probability of immature females of CL shorter than 18 mm: $P(CL_{immature}<18 \text{ mm}) = 0.03$. The likelihood of getting a representative sample of this size category is high for the population of immature individuals where the chance of finding smaller and immature individuals is extremely high. Results like the one above: $P(CL_{immature}<18 \text{ mm}) = 0.03$, i.e. low probability of smaller and immature individuals in the sample of immature lobsters will arise if smaller individuals are not adequately considered in the sampling process. To solve this under-sampling issue, theoretical exponential probability distributions of immature female CLs were built, considering information obtained from the probability distributions of the mature ones, as described below.

A non-negative random variable follows the exponential model with parameter ß > 0 [β is the inverse of the scale parameter b (expected value)], if its probability density function (eq. 3.1) and cumulative distribution function (eq. 3.2) are, respectively, the following:

$p(x) = ße^{-ßx}$ (eq. 3.1)

$P(X \leq x) = 1 - e^{-ßx}$ (eq. 3.2)

A schematic of the approach is then shown in Figure 3.1. For the purposes of building the theoretical exponential distributions, the cumulative probability of finding any immature female longer than the mature critical point CP_{mature} was defined as $P(CL_{immature} > CP_{mature}) = 0.01$ (i.e. an extremely low cumulative probability). Based on the reciprocal of this cumulative probability: $P(CL_{immature} \leq CP_{mature}) = 0.99$, the parameter ß of the exponential model was estimated using eq. 3.2 and theoretical exponential distributions were built, along with simulations of theoretical frequency distributions of immature females (Figure 3.2) for the same annual samples from which probability distributions of mature females were built. The critical point ($CP_{immature}$) was estimated by the function 'qexp()' of the R-Base package (R Core Team, 2020), such that the cumulative probability of any immature female with carapace length (=$CL_{immature}$) longer than this critical value was $P(CL_{immature} > CP_{immature}) = 0.05$, i.e., $CP_{immature}$ was defined as a cut-off for female CLs above which is not common to find any immature individuals (Figure 3.1).

3.2.1.4. TSOM estimation

$CP_{immature}$ and CP_{mature} determine three intervals of carapace lengths in the set of positive real numbers: [0, $CP_{immature}$], ($CP_{immature}$, CP_{mature}) and [CP_{mature}, +∞) (Figure 3.1). According to the definition of the maturity critical points, immature and mature female carapace lengths would be unlikely to occur in the range of the second interval above. It was assumed that the interval ($CP_{immature}$, CP_{mature}) refers to a transitional period from immature to mature. This interval was

defined as the region where it is most likely to find the theoretical size at the onset of maturity and was termed the TSOM interval (Figure 3.1). The theoretical size at the onset of maturity was defined as the single point inside the TSOM interval such that $P(CL_{immature} < TSOM) = P(CL_{mature} > TSOM)$, i.e., the cumulative probabilities of any immature (or mature) female with carapace length, shorter (or longer) than TSOM respectively, are the same. Although this can be estimated arithmetically, to avoid complex mathematical manipulations and observing the different forms of probability distribution fitted to the frequency data of mature females, the 'TSOM' was estimated in practice using an iterative process. Here, the levels of significance of both immature and mature critical points were gradually and simultaneously reduced until the TSOM interval had collapsed into the theoretical size at the onset of maturity. Thus, defining P_j and $CP_{j\ immature}$ / $CP_{j\ mature}$ respectively, as the cumulative probability and the redefined immature and mature critical points at the iterations: 1, 2, 3, …, j. This was carried out by the functions 'quantile()' and 'qexp()' mentioned above, where $CP_{immature}$ and CP_{mature} were redefined by gradually and simultaneously reducing the cumulative probabilities P_j ($CL_{immature} > CP_{j\ immature}$) and P_j ($CL_{mature} < CP_{j\ mature}$) by 0.0000001 each iteration, until the difference between these two critical points became zero ($CP_{j\ mature} - CP_{j\ immature} = 0$).

Using the method above, TSOM intervals were built for each FU / year in the time series (= 'annual TSOMs' per FU).

3.2.1.5. Sensitivity test

The sensitivity of TSOM estimation to different assumptions used for setting up the parameter β of the theoretical exponential distribution of immature female CLs, as well as the critical points of the probability distributions of immature and mature female CLs, was investigated for one fishing ground / year, i.e. FU22 (The Smalls) in 2018. Two different assumptions were considered for the critical points of the probability distributions of immature and mature female CLs: (i) $P(CL_{mature} < CP_{mature}) = P(CL_{immature} > CP_{mature}) = 0.05$; and (ii) $P(CL_{mature} < CP_{mature})$

= $P(CL_{immature} > CP_{mature})$ = 0.01. The assumption used for setting up the β parameter of the theoretical exponential distribution of immature female CLs varied according to the supposition (in this study) that the cumulative probability of any immature female with carapace length (=$CL_{immature}$) longer than the mature critical point '$P(CL_{immature} > CP_{mature})$' is extremely low. Thus, $P(CL_{immature} > CP_{mature})$ was considered always less than 0.05 and assumed the following values: 0.001; 0.002; 0.005; 0.02; 0.03 and 0.04. It is important to highlight that, for the assumption (ii) above, along with the following ones used to set up the β parameter of the theoretical exponential distribution: $P(CL_{immature} > CP_{mature})$ = 0.02, 0.03 or 0.04, there was an inversion in the extremes of the TSOM interval [CP_{mature}, $CP_{immature}$], i.e., $CP_{immature} > CP_{mature}$. Thus, in these cases, the iterative process for TSOM estimation was done by gradually and simultaneously increasing the levels of significance of both immature and mature critical points, instead of reducing them, until the TSOM interval had collapsed into the theoretical size at the onset of maturity. The coefficient of variation (ratio between the standard deviation and average values of the set of estimates in the sensitivity test) for β parameter estimates, the range of the TSOM interval and the TSOM itself were then calculated.

3.2.2. Size at maturity (L_{50}) and smallest berried female (SBF)

For the purposes of analysing the adequacy of the new metric concerning sequential maturity events over time, comparisons were made between metrics representing (i) onset of maturity (i.e. TSOM), (ii) mature (i.e. L_{50}) and (iii) berried females (i.e. smallest berried female). The L_{50} and SBF were obtained for each FU / year in the time series. SBFs were obtained by subsetting the berried females and then applying the function 'min()' from R-Base package (R Core Team, 2020) that gives the minimum value into any dataset. The average size SBF (mm, CL) for each annual sample, as well as proportions of berried and mature females that were shorter than the TSOM estimates were calculated. L_{50} estimates were obtained from maturity ogives produced from the proportion of mature individuals in each length class by logistic

regression model (glm, family binomial, 'logit' link function) using the R package FSA (Ogle *et al.,* 2020). Only models that presented a good fit to the logistic curve (i.e. well defined and complete ogives, Supplementary Figures A3.7-3.12 in appendix 2) were included.

3.2.3. TSOM relationship with average carapace length and L_{50}

The relationship between estimated TSOM and average carapace length per FU / year was investigated by fitting a linear model with the average carapace length of all females (i.e. immature and mature) as the independent variable and TSOM as the dependent one. The TSOM metric was also linked with the traditional maturity metric L_{50} by fitting a linear model, this time with TSOM as the independent variable, which was then used to predict the L_{50} metric. These models were then used to predict TSOM and L_{50} values when the methods described in the previous sections did not provide an adequate outcome (due to issues for a limited number of FU / years in the fitting of the probability distributions of mature females and / or the logistic model used for L_{50} estimation). In addition, simulations were carried out to confirm the existence of a linear relationship between TSOM and L_{50} (details in Supplementary Methods A3.1 in appendix 2).

3.2.4. TSOM versus population density

The hypothesis of a potentially inverse relationship between TSOM and population density was investigated by fitting a linear model with TSOM estimated per FU / year as the dependent variable and population density per FU / year as the independent variable. Population density estimates (Supplementary Table A3.1 in appendix 2) were obtained from annual underwater television (UWTV) surveys (Aristegui *et al.*, 2018a, b; Clements *et al.*, 2018; Doyle *et al.*, 2018a, b, c).

3.2.5. Estimation of L_{50} metric using regression analysis

Annual L_{50} estimates obtained in the present study according to the traditional method (logistic regression) were compared with annual L_{50} estimates obtained by regression analysis using two different approaches, which do not require any data about the maturity stage of the individuals, as follows: (i) by combining eq. 3.3 and 3.4 that requires as input only the mean carapace length of female samples, and (ii) by combining eq. 3.6 and 3.4 that requires as input only the population density of the FU being analysed. Bias-corrected 95 % bootstrap confidence intervals (10,000 runs) were estimated in each case using the R package boot (Canty and Ripley, 2019).

3.3. Results

3.3.1. Theoretical size at the onset of maturity (TSOM) estimates

TSOM estimates from annual samples varied from the lowest CL values of 18.4 mm seen at The Smalls (FU22) in 2002 to largest values of 33.7 mm CL at Porcupine Bank (FU16) in 2007 (Table 3.2). TSOM estimates at Porcupine Bank presented the greatest annual variability, with estimates ranging from 23.1- 33.7 mm CL (10.6 mm difference), while Western Irish Sea was the functional unit with the least annual variability, ranging from only 18.8 - 20.9 mm CL (2.1 mm difference). The other FUs presented moderately ranging annual TSOM estimates with differences of between 4 mm and 6.6 mm CL between minimum and maximum values. Some annual samples were not considered in the analysis due to issues in fitting the probability distribution i.e. inconsistencies related to the lower tail of the distribution (see q-q plots in Supplementary Figures A3.1-3.6 in appendix 2), which is extremely important for the estimation of TSOM. In such cases, TSOMs were predicted from the linear relationship between TSOM and mean CL of females derived in this study (see below). These annual

samples were the following: FU16 (2011, 2012 and 2017); FU17 (2006, 2010 and 2017); FU19 (2002) and FU22 (2006) and are indicated using grey highlighted values in Table 3.2.

3.3.2. Sensitivity test

The sensitivity test showed that the parameter β of the theoretical exponential distribution of immature female CLs and the range of the TSOM interval were very sensitive to the different assumptions used in the sensitivity test (Table 3.3). The parameter β ranged from 0.14 to 0.33. The shortest TSOM *interval* ([$CP_{immature}$, CP_{mature}]) was [21.1, 22.7] with a range of 1.6 mm CL, while the longest was [9.8, 22.7] with range of 12.9 mm CL. The coefficient of variation (CV) of parameter β of the theoretical exponential distribution of immature female CLs and the range of the TSOM interval were 30.4 and 55.3 %, respectively. However, the TSOM estimates themselves were less sensitive to the different assumptions ranging from 19.1 to 22.4 mm CL with a CV of 5.7 %. Thus, TSOM estimates from this exercise were considered stable, ≈ 19-22 mm CL, and furthermore, the TSOM estimate in 2018 at The Smalls lay approximately at the midpoint of this range (≈ 21 mm CL).

3.3.3. Size at maturity (L_{50}) and smallest berried female (SBF)

L_{50} estimates varied from 20.4 mm CL in Aran Grounds (FU17) and South Coast (FU19) in 2007 and 2018 respectively, to 32.2 mm CL in Porcupine Bank (FU16) in 2007 (Table 3.2). L_{50} estimates presented greatest annual variability in South Coast grounds with estimates ranging from 20.4 - 31.9 mm CL (11.5 mm difference), while Labadie, Jones and Cockburn Bank was the functional unit with least annual variability in L_{50} ranging from 24.3 - 27.7 mm CL (3.4 mm difference). The other FUs presented annual estimates varying from 3.8 mm - 9.0 mm CL difference between maximum and minimum values.

Individual SBF estimates were highly variable, ranging from 15 mm CL in Western Irish Sea (FU15) in 2003 to 37 mm CL in Porcupine Bank (FU16) in 2009 (Table 3.2), but the average

SBF ranged far less, from 24.1 - 42.7 mm CL across the FUs (Table 3.4). The proportion of berried females shorter than the TSOM was mostly ≤ 2 % across the FUs (Table 3.4, except for FU15 in 2003 (4.1 %), FU19 in 2005 and 2008 (25 and 2.8 %, respectively) and FU22 in 2008 (11.5 %), while the proportion of mature females (all maturity stages plus berried females) shorter than the TSOM was always insignificant across all FUs (≤ 1 %).

3.3.4. TSOM relationship with average carapace length and L_{50}

There was a significant linear relationship between estimated TSOM and average carapace length in female *Nephrops* across FU / years (i.e. including all maturity stages; F = 348.1, R^2 = 0.84, p < 0.001, Figure 3.3, Supplementary Table A3.2 in appendix 2). This relationship took the form of the equation below:

$TSOM = 3.15 + 0.63CL$ (eq. 3.3)

Similarly, there was a significant positive relationship between L_{50} and TSOM in females across FU / years (F = 45.02, R^2 = 0.40, p < 0.001, Figure 3.4, Supplementary Table A3.3 in appendix 2). This relationship took the form of the equation below:

$L_{50} = 10.97 + 0.63TSOM$ (eq. 3.4)

The equations above were used to predict TSOM and L_{50} values when it was not possible to obtain these estimates due to issues in fitting the probability distribution of matures and / or the logistic model for L_{50} estimation. This arose for the following cases: Porcupine Bank (FU16) in 2007-8, 2011-13, 2015 and 2017; Aran Grounds (FU17) in 2002, 2006, 2010 and 2017; South Coast (FU19) in 2002, 2004 and 2007; Labadie, Jones and Cockburn Bank (FU2021) in 2011-13, 2015 and 2018; and The Smalls (FU22) in 2002, 2004 and 2006 (see Table 3.2 for details).

Simulations confirmed that there is a significant linear relationship between TSOM and L_{50} (F = 638.4, R^2 = 0.87, p < 0.001, Supplementary Figure A3.13, Supplementary Table A3.4 in

appendix 2). The relationship between the simulated TSOM and L_{50} took the form of the equation below:

$L_{50} = -1.37 + 1.16\text{TSOM}$ (eq. 3.5)

3.3.5. TSOM versus population density

A linear model revealed a significant inverse relationship between TSOM in females and population density across the FU / years ($F = 113.4$, $R^2 = 0.60$, $p < 0.001$, Figure 3.5, Supplementary Table A3.5). This relationship took the form of the equation below:

$TSOM = 24.35 - 4.93Density$ (eq. 3.6)

3.3.6 Estimation of L_{50} metric using regression analysis

The L_{50} estimates derived from regression analysis using eq. 3.3, 3.4 and 3.6 above (i.e. obtained independently of any information about female maturity stage), were consistent with the estimates obtained from traditional length-maturity ogives across all the FUs (details in Table 3.5). The bias-corrected 95 % confidence intervals of estimates from regression analysis overlapped the ones from length-maturity ogives (details in Table 3.5), indicating that there was no significant difference among the sets of annual estimates obtained by the various methods.

3.4. Discussion

The primary objective of this study was to develop a methodology for estimation of the size at onset of maturity in *Nephrops*, that avoids some of the issues with existing measures. The proposed method is a theoretical size at the onset of maturity (TSOM), which is very simple, can be easily implemented, and provides alternative information from other physiological-based metrics. TSOM is built using length-maturity data, therefore it is basically a physiological maturity metric, however it can be extrapolated to length-frequency data (i.e. when no maturity readings are available). This was done in the present study through eq. 3.3

($TSOM = 3.15 + 0.63CL$) that has high predictive power ($R^2 = 0.84$), and requires as input only the mean CL of the female samples without any requirement to distinguish maturity stages.

Before discussing advantages in detail, we first discuss how the TSOM behaves in relation to other maturity metrics. First, it is important to acknowledge that maturity is a process and that there is a 'relational order' among various maturity metrics, where 'onset of maturity' events, including TSOM, could be expected to precede 'berried female' events or L_{50} events (the point at which 50 % of individuals in a sample are mature). Another distinction we can make in maturity metrics is whether their basis is in physiology, such as TSOM, similarly gonad status/presence of berried individuals (smallest berried females - SBF), and related metrics such as L_{50}. Or, whether the metric relies on indirect data such as allometric growth, in the case of morphological maturity like MSOM. Morphometric estimates of maturity like MSOM are indirect, therefore, although these are useful in the respect that they can be carried out year-round, these measures *de facto* contain major assumptions. For this reason alone, it is important to note that we do not necessarily expect morphometric and physiological maturities to be synchronized (Waiho *et al.*, 2017).

Dealing with physiological maturity measures (i.e. those listed above and the focus of the present study), we believe that these also represent different snapshots in time during the process of maturation in *Nephrops*, thus it is also not possible to absolutely compare them (see also Haig *et al.*, 2016). Annual TSOMs developed in the present study literally represent the 'onset' of maturity, as they are based on critical regions in the probability distributions of mature and immature females. They are an approximation of the carapace length at the age when the stock starts the process of maturation, a transition point from the immature to the mature stock. Whereas, other physiological-based estimates are either related to the first spawning of the stock (SBF), or when half of the population are mature (L_{50}) rather than early

maturers. Given this lengthy preamble, what trends did we notice in maturity estimates derived by these different measures?

Concerning the relational order described above, most of the TSOMs were lower than the L_{50} estimates at each FU (Table 3.2). Only 8 out of 91 TSOM estimates (≈ 8.8 % of the total) were higher than the corresponding L_{50} estimates. This occurred at Porcupine Bank (FU16) in 2007-9, 2012 and 2014 with TSOM / L_{50} estimates of 33.7 / 32.2, 31.0 / 30.5, 32.8 / 29.9, 30.6 / 27.6 and 28.2 / 23.5 mm CL, respectively; Aran Grounds (FU17) in 2010 with estimates of 22.5 / 22.4 mm CL; South Coast (FU19) in 2018 with estimates of 23.5 / 20.4 mm CL and Labadie, Jones and Cockburn Banks (FU2021) in 2016 with estimates of 26.6 / 26.0 mm CL. These cases of inversion in the relational order between TSOM and L_{50} occurred mostly at FU16 (62.5 % of the cases), perhaps because at these grounds, metrics for several FU / years were estimates based on linear regression (grey values in Table 3.2), which introduced an additional source of error. Another explanation could be related to a negative bias caused by underrepresented immature individuals in the samples used to generate L_{50} estimates in certain FU / years (Fonteles-Filho, 1989), however, this issue cannot be elucidated without further investigation. Initially, some SBFs seem to be inconsistent with this relational order, since they were lower than the TSOMs, but it is important to emphasize that SBF represents the carapace length of a single 'freakily small' mature individual among thousands in the distributions of mature females at each FU, and they are not necessarily representative of the stock. Indeed, it was the case that the proportion of SBFs shorter than the TSOMs were always insignificant (≤ 1 %) across the annual samples of thousands of mature individuals at each FU (Table 3.1). In addition, when the yearly mean of berried females at each FU (Table 3.4) was considered instead, the annual TSOM estimates were lower than those summary measures in 98 % of cases. In any case, the size of maturity must define the sexual maturity of the spawning stock rather than any one individual in this stock (Fonteles-Filho, 1989). Observing this, we propose that

TSOM estimates are more representative of the maturity of the FUs (stocks) than SBFs, since TSOMs are based on probability distributions and use all available data on mature females.

The L_{50} estimates in this study (20.4 - 32.2 mm CL) corroborate and overlap considerably with L_{50} estimates reported for females at different sites in the north-eastern Atlantic that ranged from 22.9 - 33.5 mm CL (de Figueiredo and Thomas, 1967; Tuck et al., 2000; McQuaid et al., 2006; Bell et al., 2013), as well as estimates in other geographical areas (22.0-50.7 mm CL) (de Figueiredo and Thomas, 1967; Morizur,1983; Sardà, 1991; Bianchini *et al.*, 1998; Relini *et al.*, 1998; Mente *et al.*, 2009; Ayza *et al.*, 2011; Bekrattou *et al.*, 2019). L_{50} estimates are larger and hence more conservative than TSOM estimates from management perspectives, in terms of estimating spawning stock biomass or cut-offs for minimum landing size. For these reasons, there would likely be resistance against usage of TSOM estimates for the above tasks, and in any case TSOM presents different information since it 'precedes' the other metrics within the immature-to-mature sequential process and represents the very 'onset' of maturity.

Despite their representing slightly different quantities, TSOM can be used to predict the L_{50} metric, since there is a direct linear relationship between the two (eq. 3.4): $L_{50} = 10.97 + 0.63TSOM$ ($R^2 = 0.40$). Perhaps more realistically, TSOM might be used be for calibration of L_{50} estimates. As TSOM represents the beginning of stock maturation, the proportion of first- and repeat-spawning individuals at this size should be insignificant (these are otherwise virtually impossible to distinguish in the field). This assumption might be used to build informative priors for calibration of L_{50} estimates in a Bayesian approach. Similarly, TSOM can help calibrate Bayesian approaches to improve on logistic regression models used for L_{50} estimation, which tend to overestimate the proportion of mature individuals in small length classes, due to an inevitable underestimation of small individuals in field sampling (Fonteles-Filho, 1989; Lowerre-Barbieri *et al.*, 2011). In addition, it is important to know the size at which the stock starts the process of maturation, since the onset of maturity often results in

physiological changes leading to distinct growth patterns between immature and mature stocks. These must also be considered in the fitting of the growth equations for stock assessment purposes (Beverton and Holt, 1957). The size at onset of sexual maturity is extremely important for understanding the reproductive strategies of a species and to calculate the reproductive output (McQuaid *et al.*, 2005). TSOM can be used to provide first approximation of these quantities when data is limited (e.g. in data-poor situations, in non-commercial species, or species of conservation importance). Essentially, TSOM fulfils a different role from the existing metrics, representing a new quantity which provides additional information on early maturation, and which does so in the absence of routine maturity measurements (see below).

The great advantage of the TSOM methodology is that it is simple, efficient and stable, as showed by sensitivity test carried out in this study (CV of 5.7 %). It is based on data routinely collected by national organizations responsible for the assessment and management of *Nephrops* stocks. ICES (2006) recommends that *Nephrops* size of maturity estimates must be based only on data collected within a specific time-window as the onset of spawning can be biased by sampling artefacts associated with seasonal breeding / burrow-dwelling cycles of the mature females (Sardà, 1991; Johnson *et al.*, 2013; Powell and Eriksson, 2013; Watts *et al.*, 2016; Santana *et al.*, 2020). Therefore, one potential issue with TSOM would be bias caused by the seasonality of the female reproductive cycle if the initial maturity data on which TSOM calculations were built were obtained at the wrong time of year. To address this, an important result in the present study was the very simple relationship that relates TSOM with the average carapace length of females (mature and immature) in the time series. This is available in this study by eq. 3.3 and 3.4 ($TSOM = 3.15 + 0.63CL$ and $L_{50} = 10.97 + 0.63TSOM$, with R^2 = 84 and 40 % respectively), which addresses issues of non-seasonal data. Indeed, when both are combined, only the mean CL of female samples (all females without distinction of maturity stage) is necessary to predict the TSOM and L_{50} metrics, as was necessary in some isolated

cases in the present study. Clearly, the latter will incorporate an error (particularly in eq. 3.4, as shown by R^2 values), however it may nevertheless be useful in data-limited contexts. Eq.3.3 is consistent with a positive relationship between SOM and mean CL previously observed in *Nephrops* by Tuck *et al.* (2000) in the Firth of Clyde. Although, similar to many fisheries data, one potential drawback of its use is where larger individuals are underrepresented in heavily exploited populations. It has been suggested in previous studies that size-selective mortality (e.g. from fishing) can induce evolutionary changes in body sizes within populations, however the extent to which these are important compared with ecological (density-dependent suppression of body size) and physiological effects (e.g. temperature-size relationships) is hotly debated (Kuparinen and Merilä, 2007; Swain *et al.*, 2007; Haig *et al.*, 2016). In *Nephrops*, little evidence of heritable selection (genetic effects) is clear from genetic structuring, with little or none seen in the Atlantic (Stamatis *et al.*, 2004; Gallagher *et al.*, 2018), likely due to highly dispersing larvae (McGeady *et al.*, 2020) and hence weak reproductive isolation. Rather, there is strong evidence for ecological size effects due to density-dependent suppression of body size, including recent evidence from tagging studies in Ireland that growth was lower in sub-ordinate males in higher density patches (Merder *et al.*, 2019). Hence, we think that ecological rather than evolutionary effects on body size metrics are relevant in this case. One question we can therefore ask is: does density also scale with size of maturity?

Related to the above question, an inverse relationship between annual TSOM and population density was found for females across Irish FUs in the present study. This was unsurprising for the reasons outlined above, and because there is a well-established inverse relationship between density and body size in *Nephrops* across EU grounds: high-density grounds have *Nephrops* of smaller body sizes (Briggs, 1995; Tuck *et al.*, 2000; Johnson *et al.*, 2013). However, although Queirós *et al.* (2013) found a similar relationship between maturity (estimated using morphometric techniques) of male *Nephrops* and population density in Scottish waters,

surprisingly, a similar trend was not observed in females. Nevertheless, eq. 3.3 of the present study indirectly supports this observation, as it scales with body size. Taken together, all these results demonstrate the necessity to manage *Nephrops* stocks in separate functional units with individual population density, body size, and maturity characteristics (see also Briggs, 1995; Queirós *et al.*, 2013).

Size of maturity is a critical parameter in stock assessment of commercially harvested fish stocks as it forms the basis of estimating the spawning stock biomass (SBB). Applications of the new TSOM method described in the present study provides a flexible measure of the SBB from length-maturity data, albeit a slightly more generous estimate (since it is lower / reached earlier) than is provided by L_{50}. We have shown how this can be extrapolated, via eq. 3.3 and 3.4, hence SSB can be estimated with no information apart from length-frequency data. Further, the negative relationship between TSOM and population density provides an approximation of the spawning stock and recruitment from underwater television surveys via the population density vs TSOM (R^2 = 60%, Figure 3.5). TSOM and its relationships with other metrics might also contribute to improvements in stock assessment and management of *Nephrops* in data-limited European FUs, e.g. those without regularly sampled length-maturity data, and might be used to calibrate more routinely used estimates e.g. L_{50}. It might even be applied to males *Nephrops* and a range of new species e.g. in macroecology settings, where a comparable size at maturity metric is required for lots of species, where length-frequency data exists, but where regular size of maturity data is limited or patchy.

3.5. Tables

Table 3.1. Functional management units (FUs) with sample sizes of mature female Nephrops considered for estimation of the theoretical size at the onset of maturity (i.e. 'TSOM') in the present study.

Functional Unit	Sample size (n)																	
	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
FU15	3329	7838	8049	7103	2753	3569	6024	3107	3230	4236	4800	8045	5793	4788	3613	5323	2140	1759
FU16	-	-	-	14	-	2	202	3601	9792	4966	11296	915	136	3251	2383	3043	879	1303
FU17	-	1606	3320	5269	2215	721	2166	1450	2922	4462	8133	4245	3830	4042	505	3987	2293	2270
FU19	-	160	288	250	1286	796	2284	2806	1627	1582	2722	2380	2807	2014	566	2528	1359	1924
FU2021	-	-	5	-	-	8	8	-	247	246	98	137	1167	242	279	2114	1596	2923
FU22	-	957	2337	2520	4336	6641	7377	2758	3146	4789	2767	4048	2757	2920	1817	2857	3063	3628

FU15 – Irish Sea West
FU16 – Porcupine Bank
FU17 – Aran Grounds
FU19 – South Coast
FU2021 - Labadie, Jones and Cockburn Banks
FU22 – The Smalls

Table 3.2. Theoretical size at onset of maturity ('TSOM'), L_{50} i.e. size class at which 50% of the females are sexually mature and smallest berried female (SBF) for female *Nephrops* at Irish FUs: Western Irish Sea (FU15), Porcupine Bank (FU16), Aran Grounds (FU17), South Coast (FU19), Labadie, Jones and Cockburn Banks (FU2021) and The Smalls (FU22). All values are given in carapace length (mm). The greyed values indicate when estimates were obtained by regression analysis (eq. 3.3,3. 4 and 3.6).

Year	FU15			FU16			FU17			FU19			FU2021			FU22		
	TSOM	L_{50}	SBF	TSOM	L_{50}	SBF	TSOM	L_{50}	SBF	TSOM	L_{50}	SBF	TSOM	L_{50}	SBF	TSOM	L_{50}	SBF
2001	19.1	23.6	17.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2002	19.6	24.0	19.0	-	-	-	21.4	24.5	22.0	21.6	24.5	24.0	-	-	-	18.4	22.6	23.0
2003	19.8	24.2	15.0	-	-	-	20.7	23.6	23.0	23.1	31.9	24.0	-	-	-	22.5	26.4	23.0
2004	20.5	24.5	17.0	-	-	-	22.1	23.9	22.0	19.8	23.4	26.0	-	-	-	25.0	26.7	21.0
2005	20.5	25.4	22.0	-	-	-	21.4	24.7	23.0	21.8	25.4	17.0	-	-	-	22.2	24.3	22.0
2006	20.3	23.1	19.0	-	-	-	19.0	22.5	17.0	25.3	28.8	23.0	-	-	-	20.2	25.7	21.0
2007	19.7	23.7	20.0	33.7	32.2	-	19.1	20.4	-	25.7	27.2	27.0	-	-	-	20.7	23.0	20.0
2008	19.7	23.8	17.0	31.0	30.5	-	21.3	24.0	24.0	25.0	26.0	22.0	-	-	-	21.3	22.4	17.0
2009	20.1	23.3	21.0	32.8	29.9	37.0	20.9	22.8	22.0	23.8	27.6	23.0	23.3	25.5	28.0	21.7	24.1	20.0
2010	19.7	22.5	22.0	29.0	29.7	27.0	22.5	22.4	17.0	25.7	29.0	26.0	21.8	24.8	25.0	23.8	24.8	16.0
2011	20.5	22.5	19.0	28.4	29.7	24.0	23.0	24.9	24.0	22.3	26.6	23.0	22.0	24.8	23.0	23.5	28.3	24.0
2012	19.1	21.6	20.0	30.6	27.6	30.0	21.6	23.7	23.0	21.7	26.5	24.0	21.1	24.3	25.0	20.9	25.6	20.0
2013	18.8	22.4	21.0	27.1	28.0	32.0	21.2	23.3	17.0	21.9	25.7	22.0	25.1	26.8	26.0	22.3	24.8	21.0
2014	20.0	22.3	19.0	28.2	23.5	-	23.0	24.9	26.0	22.6	26.8	21.0	22.9	27.7	27.0	23.0	25.3	23.0
2015	20.9	22.9	21.0	25.7	27.2	-	22.1	27.2	23.0	22.2	31.0	23.0	22.8	25.3	28.0	21.6	24.7	23.0
2016	19.8	22.7	20.0	24.4	27.5	24.0	22.2	24.9	21.0	24.4	28.0	24.0	26.6	26.0	25.0	20.7	23.3	20.0
2017	19.6	22.1	20.0	23.3	25.6	25.0	21.6	24.6	22.0	23.5	27.7	26.0	22.8	27.7	24.0	20.7	22.4	21.0
2018	19.4	23.3	20.0	23.1	23.2	22.0	20.8	22.2	22.0	23.5	20.4	25.0	23.9	26.0	26.0	21.1	22.9	21.0

Table 3.3. Sensitivity test for the TSOM estimate in the Smalls (FU22) in 2018, considering different assumptions for setting up the β parameter of the theoretical exponential distribution of CLs of immature females [$P(CL_{immature} > CP_{mature})$] and the immature and mature critical points [$P(CL_{immature} > CP_{immature})$ and $P(CL_{mature} < CP_{mature})$, respectively]. The assumption and TSOM estimate used in the present study for the above FU / year (FU22 / 2018) are indicated by an (*). Coefficients of variation (ratio between the standard deviation and average value of the set of estimates in the sensitivity test) are provided for the parameter β, the range of TSOM interval and TSOM itself.

$P(CL_{mature} < CP_{mature})$ and $P(CL_{immature} > CP_{immature})$	$P(CL_{immature} > CP_{mature})$	β	$CP_{immature}$	CP_{mature}	TSOM
0.05	0.01*	0.20	14.8	22.7	21.1*
0.05	0.001	0.30	9.8	22.7	19.5
	0.002	0.27	10.9	22.7	19.9
	0.005	0.23	12.8	22.7	20.5
	0.02	0.17	17.4	22.7	21.7
	0.03	0.15	19.4	22.7	22.1
	0.04	0.14	21.1	22.7	22.4
0.01	0.001	0.33	13.8	20.7	19.1
	0.002	0.30	15.4	20.7	19.6
	0.005	0.26	18.0	20.7	20.2
	0.02	0.19	24.4	20.7	21.4
	0.03	0.17	27.2	20.7	21.8
	0.04	0.16	29.7	20.7	22.1
Coefficient of variation		β	Range of TSOM *interval*		TSOM
		30.4 %	55.3 %		5.7 %

Table 3.4. Mean smallest berried female (SBF in mm, carapace length) and percentage of berried females shorter than theoretical size at the onset of maturity (TSOM) across Irish functional units: Western Irish Sea (FU15), Porcupine Bank (FU16), Aran Grounds (FU17), South Coast (FU19), Labadie, Jones and Cockburn Banks (FU2021) and The Smalls (FU22). NAs indicate that there were no berried females in the respective FU / year

Year	FU15		FU16		FU17		FU19		FU2021		FU22	
	Mean	< TSOM (%)	Mean	< TSOM (%)	Mean	< TSOM (%)	Mean	< TSOM (%)	Mean	< TSOM (%)	Mean	< TSOM (%)
2001	24.8	0.3	-	-	-	-	-	-	-	-	-	-
2002	25.2	0.6	-	-	31.5	0	27.4	0	-	-	30.7	0
2003	25.5	4.1	-	-	29.4	0	31.0	0	-	-	28.6	0
2004	26.5	0.3	-	-	29.7	0.6	31.0	0	-	-	31.9	1.7
2005	26.5	0	-	-	30.5	0	26.9	25.0	-	-	30.0	0.2
2006	25.3	1.3	-	-	24.1	0	34.1	1.6	-	-	27.7	0
2007	26.5	0	NA	NA	NA	NA	31.7	0	-	-	26.5	0.6
2008	25.6	0.8	NA	NA	29.7	0	32.6	2.8	-	-	25.6	11.5
2009	26.8	0	42.7	0	28.6	0	32.5	0.7	31.0	0	28.9	0.8
2010	26.5	0	40.9	1.3	28.8	0	32.7	0	30.5	0	29.8	1.7
2011	25.3	1.0	39.5	0	33.9	0	32.1	0	25.0	0	30.8	0
2012	24.9	0	41.7	0	27.9	0	32.4	0	27.5	0	30.1	0.3
2013	26.5	0	41.9	0	29.6	1.3	31.1	0	30.4	0	30.7	0.5
2014	25.4	0.6	NA	NA	30.9	0	31.3	1.0	30.4	0	31.0	0.4
2015	26.0	0	NA	NA	31.5	0	33.2	0	30.8	0	28.5	0
2016	25.4	0	33.6	0.25	32.5	1.0	32.4	1.4	32.7	1.9	28.6	1.2
2017	25.3	0	34.5	0	31.4	0	31.0	0	32.1	0	28.8	0
2018	26.4	0	30.5	0.16	30.1	0	32.5	0	33.8	0	28.0	0.7

Table 3.5. L_{50} estimates (mm CL) for female *Nephrops* at Irish FUs: Western Irish Sea (FU15), Porcupine Bank (FU16), Aran Grounds (FU17), South Coast (FU19), Labadie, Jones and Cockburn Banks (FU2021) and The Smalls (FU22) obtained in this study from length-maturity data by logistic regression (maturity ogives, L_{50}) and linear regression (L_{50}' and L_{50}'') without requirement for distinction of maturity stage through two different approaches: (i) by combing eq.3.3 and 3.4 (derived in this study) with mean CL (mm) as input for the L_{50} metric estimation and (ii) by combining eq. 3.6 and 3.4 (derived in this study) with population density (burrows/m^2) as input for the L_{50} metric estimation. Bias-corrected 95 % confidence intervals are provided for comparisons of the different estimates (L_{50}, L_{50}' and L_{50}'')

Year	FU15			FU16			FU17			FU19			FU2021			FU22		
	L_{50}	L_{50}'	L_{50}''	L_{50}	L_{50}'	L_{50}''	L_{50}	L_{50}'	L_{50}''	L_{50}	L_{50}'	L_{50}''	L_{50}	L_{50}'	L_{50}''	L_{50}	L_{50}'	L_{50}''
2001	23.6	23.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2002	24.0	23.3	-	-	-	-	-	24.5	23.9	-	24.5	-	-	-	-	-	22.6	-
2003	24.2	23.4	23.2	-	-	-	23.6	24.0	23.4	31.9	25.5	-	-	-	-	26.4	25.1	-
2004	24.5	23.9	23.2	-	-	-	23.9	24.9	23.0	-	23.4	-	-	-	-	-	26.7	-
2005	25.4	23.9	23.1	-	-	-	24.7	24.5	23.8	25.4	24.7	-	-	-	-	24.3	25.0	-
2006	23.1	23.8	23.3	-	-	-	22.5	23.0	24.9	28.8	26.9	25.7	-	-	24.9	25.7	23.7	24.8
2007	23.7	23.4	23.4	-	32.2	-	20.4	23.0	24.2	-	27.2	-	-	-	-	23	24.0	25.2
2008	23.8	23.4	23.9	-	30.5	-	24.0	24.4	25.0	26.0	26.7	-	-	-	-	22.4	24.4	25.2
2009	23.3	23.6	23.7	29.9	31.6	-	22.8	24.1	24.7	27.6	26.0	-	25.5	25.6	-	24.1	24.6	25.2
2010	22.5	23.4	23.5	29.7	29.2	-	22.4	25.2	24.4	29.0	27.2	-	24.8	24.7	-	24.8	26.0	25.2
2011	22.5	23.9	23.6	29.7	28.9	-	24.9	25.5	24.7	26.6	25.0	25.3	-	24.8	-	28.3	25.8	25.0
2012	21.6	23.0	23.5	27.6	22.4	25.8	23.7	24.6	25.3	26.5	24.6	25.4	-	24.3	24.5	25.6	24.1	24.8
2013	22.4	22.8	23.9	-	28.0	26.0	23.3	24.3	25.3	25.7	24.8	25.5	-	26.8	25.8	24.8	25.0	25.0
2014	22.3	23.6	23.7	23.5	28.7	26.0	24.9	25.5	25.4	26.8	25.2	25.3	27.7	25.4	25.7	25.3	25.5	24.7
2015	22.9	24.1	23.9	-	27.2	-	27.2	24.9	25.1	31.0	25.0	25.6	-	25.3	25.7	24.7	24.6	24.8
2016	22.7	23.4	23.7	27.5	26.3	25.9	24.9	25.0	25.4	28.0	26.3	25.7	26.0	27.7	25.8	23.3	24.0	25.3
2017	22.1	23.3	23.5	-	25.6	26.0	-	24.6	25.3	27.7	25.8	25.5	27.7	25.3	24.9	22.4	24.0	24.6
2018	23.3	23.2	23.2	23.2	25.5	25.9	22.2	24.1	25.1	20.4	25.8	26.0	-	26.0	25.5	22.9	24.3	25.3
	Bias-corrected 95% confidence interval																	
L_{50}	(22.8, 23.7)			(24.8, 29.1)			(22.9, 24.5)			(25.6, 28.5)			(25.2, 27.7)			(23.8, 25.4)		
L_{50}'	(23.3, 23.6)			(26.4, 29.5)			(24.1, 24.8)			(25.1, 26.1)			(25.1, 26.4)			(24.2, 25.1)		
L_{50}''	(23.4, 23.7)			(25.9,26.0)			(24.2, 24.9)			(25.4, 25.7)			(25.0, 25.6)			(24.9, 25.1)		

3.6. Figures

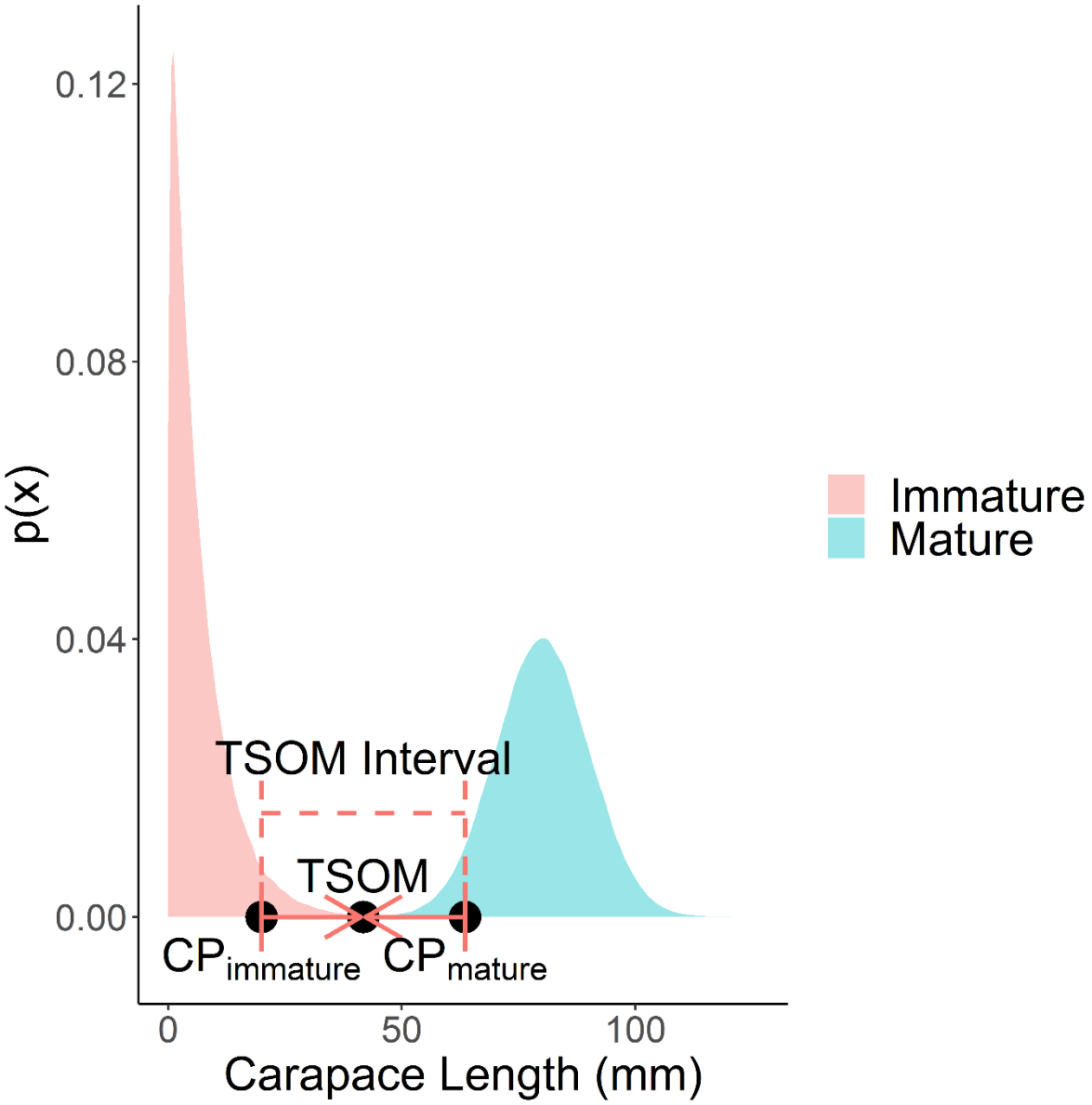

Figure 3.1. Explanatory plot showing probability distribution of mature female *Nephrops* (CL, mm), theoretical probability distribution of immature female *Nephrops*, critical points (CPs), TSOM interval, and theoretical size at the onset of maturity (TSOM) after the TSOM interval has been collapsed. P (x) is the probability density of carapace lengths.

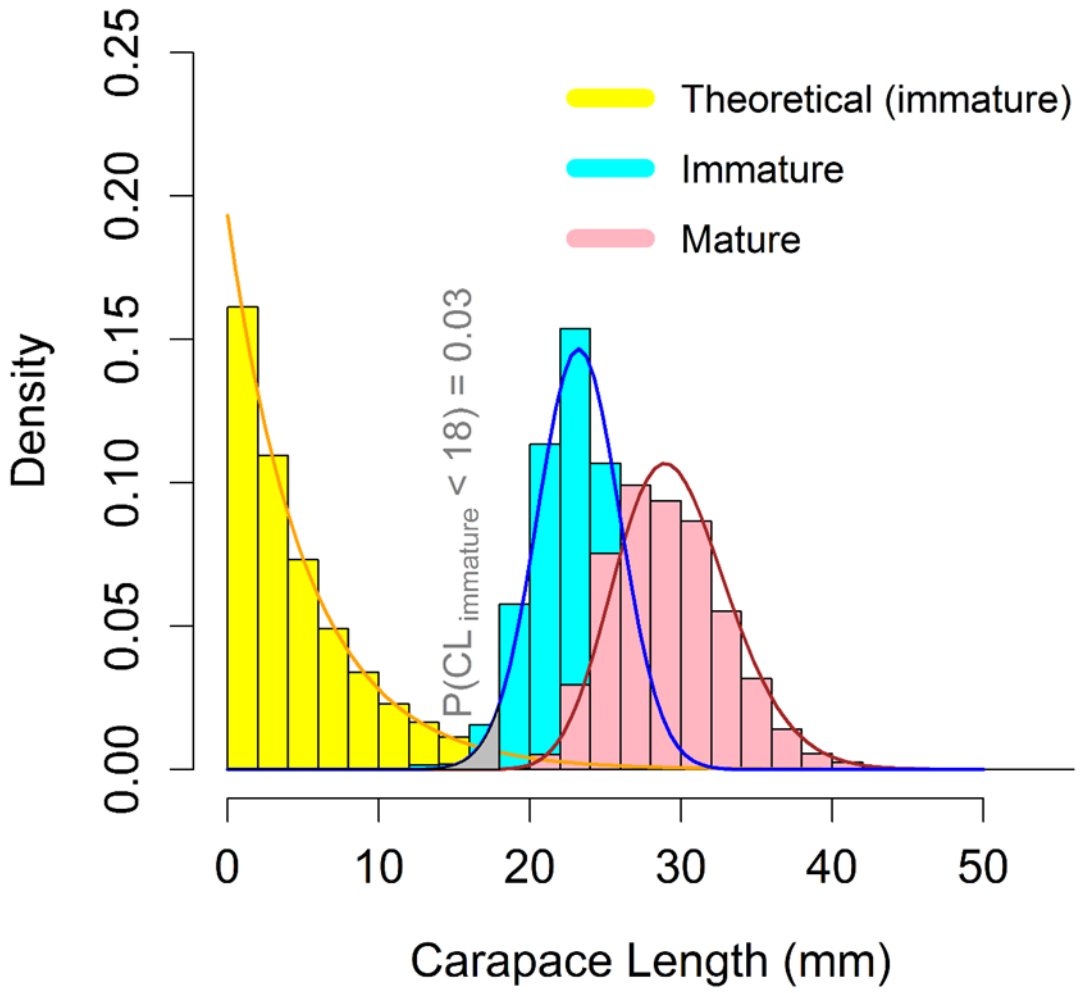

Figure 3.2. Plot showing frequency distributions of immature and mature female *Nephrops* of The Smalls (FU22) with their respective fitted probability density curves, the theoretical frequency distribution of immature female *Nephrops* with fitted exponential curve and area under the density curve of immature female *Nephrops*, indicating the probability of female *Nephrops* with carapace length less than 18 mm CL.

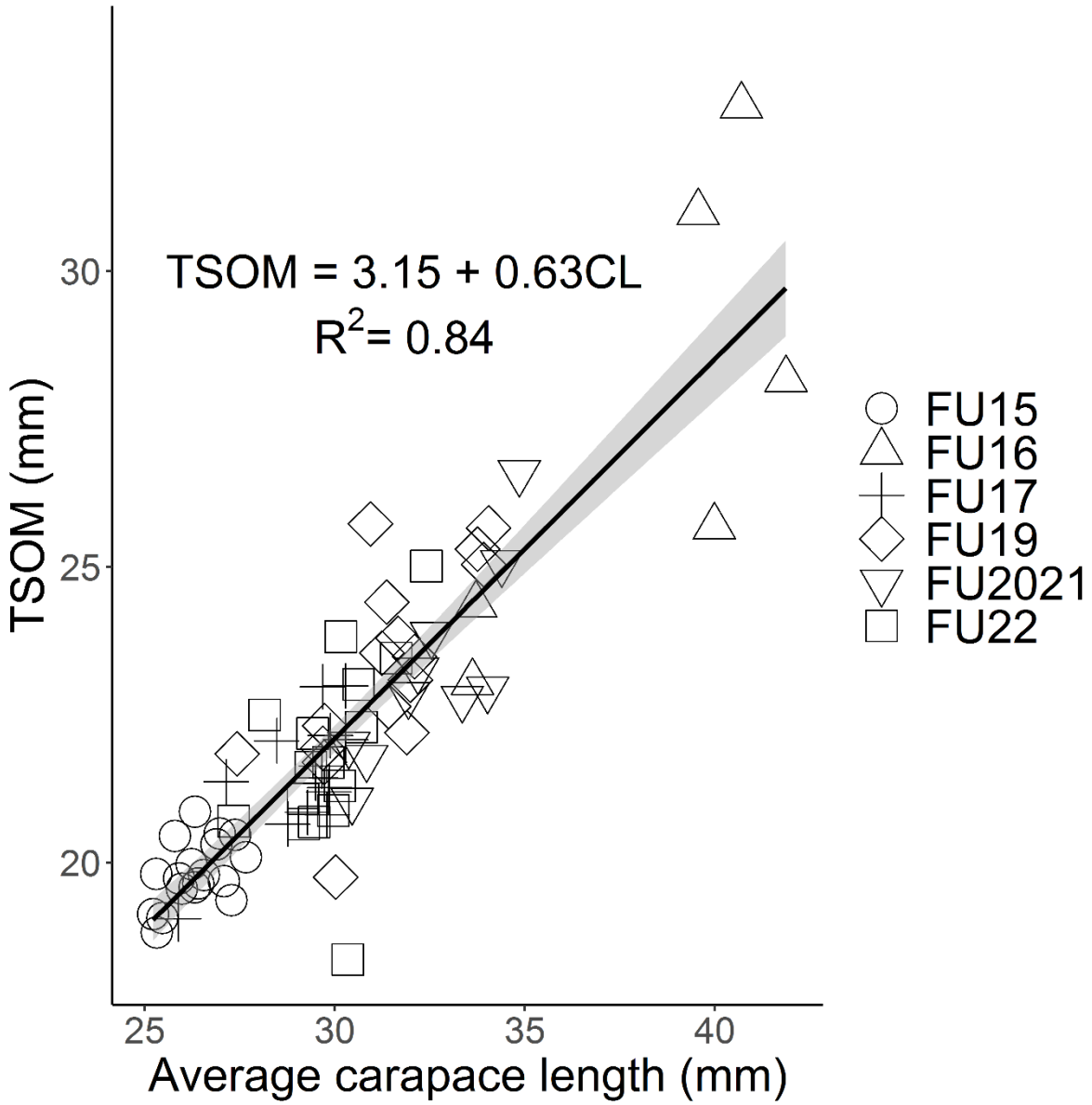

Figure 3.3. Linear regression model showing a significant positive relationship between female *Nephrops* TSOM (CL, mm) and the mean carapace length (mm) of all female *Nephrops* (immature and mature) in the sample, with annual values per FU presented for Western Irish Sea (FU15), Porcupine Bank (FU16), Aran Grounds (FU17), South Coast (FU19), Labadie, Jones and Cockburn Banks (FU2021) and The Smalls (FU22). 95% confidence interval of the predicted values in grey.

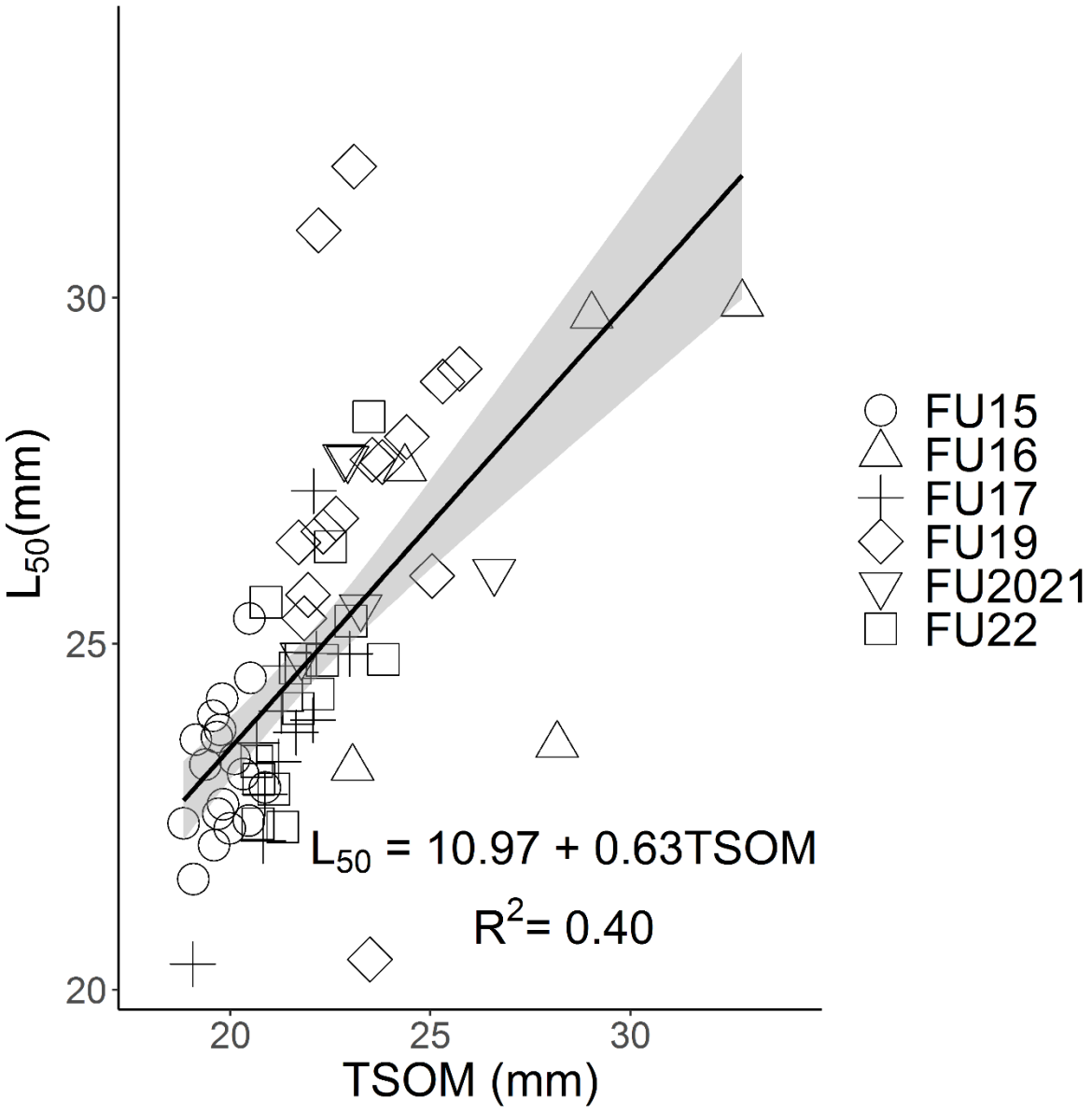

Figure 3.4. Linear regression model showing a significant positive relationship between female *Nephrops* L_{50} and TSOM (CL, mm) estimated from length-maturity data in this study, with annual values per FU presented for Western Irish Sea (FU15), Porcupine Bank (FU16), Aran Grounds (FU17), South Coast (FU19), Labadie, Jones and Cockburn Banks (FU2021) and The Smalls (FU22). 95% confidence interval of the predicted values in grey.

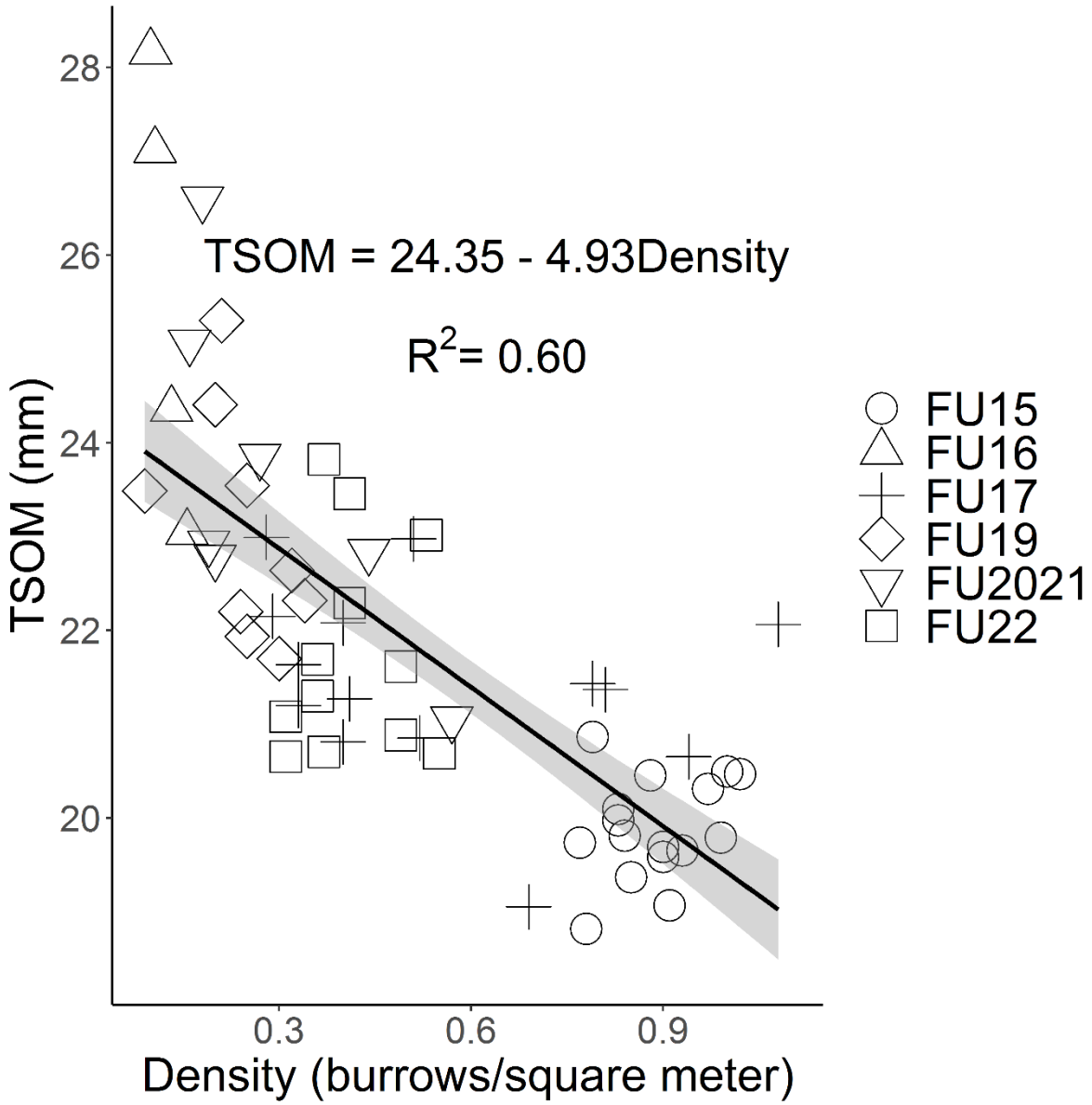

Figure 3.5. Linear regression model showing a significant inverse relationship between female *Nephrops* TSOM (CL, mm) and population density (ind. m-2), with annual values per FU presented for Western Irish Sea (FU15), Porcupine Bank (FU16), Aran Grounds (FU17), South Coast (FU19), Labadie, Jones and Cockburn Banks (FU2021) and The Smalls (FU22). 95% confidence interval of the predicted values in grey.

Chapter 4

Tools to examine *Nephrops*' morphometric maturity

4. Morphometric size at the onset of maturity and discrimination of *Nephrops norvegicus* (Linnaeus, 1758) populations across a gradient of population density in the northeast Atlantic

Author contributions: A.M.P. and C.A.S.S. designed the study and carried out the statistical analysis; A.M.P. and C.A.S.S. wrote the manuscript, prepared the figures and reviewed the manuscript. C.A.S.S., A.W and C.S. carried out the lab work. A.M.P. and C.A.S.S. obtained the funding.

Abstract

Morphometric techniques can be used in the assessment and management of fishery resources, for example, in the estimation of population parameters such as the size at the onset of maturity or on the identification and characterization of fish stocks (stock identification). The main aim of the present study was to estimate the morphometric size at onset of maturity (MSOM) of male and female *Nephrops* across Irish and Scottish functional units and to test the hypothesis of a potential inverse relationship between these estimates and population density on these grounds. Secondary objectives were to examine possible morphometric discrimination of *Nephrops* populations across the functional units in the study and, if this exists, to test whether it can be linked to density on the fishing grounds. MSOM was estimated, based on different body structures of males and females, including the male first pleopod, which was used for this purpose for the first time. A series of morphometric variables were discovered to have significant allometric growth relationships that were potentially indicative of sexual maturity (i.e. were potential secondary sexual characteristics). However, due to the great variability in these MSOM estimates, mainly across different body structures but also between years within grounds, it was difficult to choose the most representative metric to represent the MSOM. Furthermore, the study was unable to identify any significant inverse relationship between

these various estimates and population density. However, it was possible to discriminate samples morphologically across the Moray Firth, Firth of Clyde, Western Irish Sea and Aran Grounds in females and between the Western Irish Sea, Aran Grounds and Moray Firth in males. Therefore, this study showed, for the first time, morphometric variation in *Nephrops* populations among a variety of functional units in the northeast Atlantic.

4.1. Introduction

Morphometric analyses have traditionally been an important tool in ecology, aiding ventures such as the taxonomic classification of species, identification and characterization of different populations and allometric relationships among body structures (Blackith and Reyment, 1971). In terms of sustainable management of valuable fishery resources, these tools have been used for the purposes of stock assessment including, the estimation of population parameters such as the size at the onset of maturity in crustaceans (Evans *et al.*, 1995; Leme, 2005; Hirose *et al.*, 2013; Haig *et al.*, 2016; Severino-Rodrigues *et al.*, 2016; Cusba and Paramo, 2017; Öndes *et al.*, 2017) and identification and characterization of stock structure in a variety of species (Elliot *et al.*, 1995; Tzeng *et al.*, 2001; Paramo and Saint-Paul, 2010; Chen *et al.*, 2015; Siddiki *et al.*, 2016; Kalate *et al.*, 2018). Besides this, the size/age at which sexual maturity is attained is also essential information for the estimation of spawning stock potential which performs a primary role in the stock assessment and management of fishery resources (Fonteles Filho, 1989; Hilborn and Walters, 1992).

Morphometric analysis has been used as an alternative to physiological-based methods in the estimation of the size at the onset of maturity (SOM) because it is easier to obtain and less prone to seasonal bias (Queirós *et al.*, 2013). The morphometric approach for estimation of SOM is based on the allometry of certain body structures (secondary sexual characters, see Hartnoll, 1974) at maturity, e. g. chelae, abdomen and first pleopod. Allometry can be defined as a disproportional increase of any body structure of an individual relative to its body size

(Bartels *et al.*, 2010). It is called positive allometry when the body structure increases faster than body size or negative allometry when the body structure increases slower than the body size (Bartels *et al.*, 2010). The morphometric approach has been extensively researched as a tool for estimating the SOM in *Nephrops norvegicus* (Farmer, 1974a; Hillis, 1981; Mori *et al.*, 1996; Tuck *et al.*, 2000; McQuaid *et al.*, 2006; Queirós *et al.*, 2013) which is the most important shellfish resource exploited in Europe with landings of 56,696 tonnes in 2017 (FAO, 2020).

One important issue for the sustainable management of *Nephrops norvegicus* (hereafter called *Nephrops*) is that the body size and SOM in this species seems to be dependent on the population density (Briggs, 1995; Tuck *et al.*, 2000; Johnson *et al.*, 2013; Queirós *et al.*, 2013; Merder *et al.*, 2019; da Silva Santana, C. A. *et al.*, 2021), and potentially on environmental conditions / habitat characteristics, such as water temperature / redox state and granulometry of the sediment at different grounds (Tully and Hillis, 1995; Maynou and Sardà, 1997; Tuck *et al.*, 1997). Density-dependent processes (e.g. suppression of size, reproductive output, etc.) which act in a negative way as stock sizes get too high (relative to the carry capacity) have implications in the management of *Nephrops* stocks because higher-density grounds may have more compensatory potential to counteract fishing (Ricker, 1954; Beverton and Holt, 1957; Merder *et al.*, 2019). This means that more fishing can take place in a stock that exists at high density as, in theory, this should serve to prevent negative density-dependent factors coming into play. Understanding these factors is of primary importance in helping to determine what the appropriate level of fishing exploitation may be. If there are large differences in density across fishing grounds, as is the case in *Nephrops*, density-dependent effects on body size and weight also has implications for minimum landing sizes, especially if this impacts on size at maturity. Leading on from this, periodic estimation of the SOM is needed, particularly if this seems to be density-dependent, because population density can fluctuate according to the

fishing pressure exerted on the fishing grounds and, hence, monitoring is required (Queirós *et al.*, 2013).

The main aim of this study was to examine a range of morphometric variables that may indicate onset of maturity (termed 'MSOM'), based on different body structures in males and females, including one structure never used before in studies about morphometric maturity, the male first pleopod that is modified for copulation. After establishing the most promising body structures for this purpose, a follow-on aim was to verify a potential inverse relationship between the 'best' morphometrical maturity metrics and population density across the grounds in the study. Such a relationship was already observed for the theoretical size at the onset of maturity across Irish grounds (da Silva Santana, C. A. *et al.*, 2021).

Secondary objectives were to examine possible morphometric discrimination of *Nephrops* populations at a variety of Irish and Scottish functional units, to identify the main characters contributing to such discrimination, before finally investigating any link with population density across these grounds. There have been previous suggestions that morphometric discrimination of *Nephrops* populations seems to be related to the sediment type (Maynou and Sardà, 1997). In addition, *Nephrops* demonstrates a preference for particular grades of sediment but only up to a threshold that results a dome-shaped response between *Nephrops* density and % silt+clay in the sediment i.e. a positive response of density up to ~60% silt+clay and a fall-off in the density response at higher silt+clay percentages (Campbell *et al.*, 2009; Johnson *et al.*, 2013, Merder *et al.*, 2019). Thus, the hypotheses tested are: (i) there is an inverse relationship between MSOM and population density across the grounds analysed in the study; (ii) *Nephrops* populations can be morphometrically discriminated across Irish and Scottish FUs and (iii) functional units with significantly different population densities present *Nephrops* populations with distinct morphometric structure (defined below), while *Nephrops* possess

similar morphometric structure at grounds which lack significantly different population densities.

Morphometric techniques are an effective tool at delineating population structure and are one of the simplest and most cost-effective tools to identify and characterize aquatic resources stocks, assemblages and populations (Chen *et al.*, 2015; Siddik et al., 2016), especially when apparently no genetic structure exists e.g. because of shared spawning grounds or weak genetic structure in open populations that is commonly seen in marine organisms. In fact, genetic studies reveal a low-level of differentiation among *Nephrops* populations across the Atlantic and Mediterranean (Maltagliati *et al.*, 1998; Passamonti *et al.*, 1997; Stamatis *et al.*, 2004; Streiff *et al.*, 2001), apart from some significant differences between northeast Atlantic and east Mediterranean populations by mitochondrial D-loop DNA markers (Gallagher *et al.*, 2018). By contrast with the genetic approach, there is a single study by Maynou and Sardà (1997) that revealed spatial morphometric variation between *Nephrops* populations in the Catalan Sea. Thus, little is known about morphological variation across the range of *Nephrops* geographical distribution and it is fair to question whether there is any morphometric variability among *Nephrops* populations in the northeast Atlantic? Understanding habitat-morphology interactions (Gomes *et al.*, 2016) is important because it may reveal a link between environmental and morphological variation. This might clarify the evolutionary relevance of morphological variations by evaluating whether any specific feature enhances the functional capability of individuals in distinct environments. In addition, strongly varying morphometric features could be used in a range of applications such as seafood traceability initiatives in the trade of live or whole *Nephrops*.

Nephrops is the most valuable crustacean species exploited across the Europe Union. The sustainability of this valuable fishery resource is of great concern for Europe Union authorities, since some FUs periodically display signs of over-exploitation (Anon., 2011; Lordan *et al.*,

2013). It is expected that this study will contribute to a more robust stock assessment and management of this species, observing 'The Marine Strategy Framework Directive and reformed Common Fisheries Policy (CFP)' that require ecosystem-based fisheries management that observes interactions among all components of the ecosystem.

4.2. Methods

4.2.1. Sample collection

Samples were supplied from the following functional units: FU15 (Western Irish Sea), FU17 (Aran Grounds) and FU22 (The Smalls) in Ireland, and from FU9 (Moray Firth) and FU13 (Firth of Clyde) in Scotland. Samples were collected by: (i) beam trawl consisting of 30 minute tows at 2.5 to 2.7 knots at 24 stations in FU15, (ii) 4m beam trawl with 20 mm liner deployed at 10 randomly selected stations in FU17 and FU22 (2017-2018), and (iii) BT 149B trawl towed at speeds of between 2 and 3 knots for periods ranging from 0.5 to 1.5 h in FU9 and FU13. Survey timings were as follows: FU15 (20-24 August 2018), FU17 (18-28 June 2017 / 19-26 June 2018), FU22 (9-17 August 2017 / 19-26 August 2018) and FU9 and FU13 (3-25 June 2018). After collection, samples were stored in freezers aboard the respective research vessels before being shipped to the laboratory for analysis. The number of *Nephrops* analysed per year / FU can be seen in Supplementary Table A4.1 in appendix 3.

4.2.2. Morphometric measurements

After thawing at room temperature, 19 / 17 morphometric measurements were taken for each male / female individual in the sample. Except for the modified first pleopod in males, all the other structures have previously been considered in *Nephrops* morphometric maturity studies (Farmer, 1974a; Hillis, 1981; Mori *et al.*, 1996; Tuck *et al.*, 2000; McQuaid *et al.*, 2006; Queirós *et al.*, 2013). All relevant body structures and measurement dimensions are shown in Table 4.1 and Figure 4.1, respectively. The measurements for the appendix masculina and male 1st pleopod were taken to the nearest 0.05 mm by optical microscope at 10x magnification. The

measurements for the other body structures in samples from FU17 and 22 in 2017 were taken to the nearest 0.05 mm by vernier calliper, while measurements from all other samples were taken with Mitutoyo electronic Calliper Absolute IP 67 (0.01 mm resolution / ± 0.02 mm accuracy). The maturity stage for females was recorded based on gonad colouration / size and presence of eggs on the abdomen (Mente et al. 2009).

4.2.3. Statistical analysis

4.2.3.1. Size at the onset of maturity from morphometric data (MSOM)

The estimation of the size at the onset of maturity was carried out on untransformed data by segment regression models as described in Queirós *et al.* (2013). Carapace length (CL) was considered the independent variable and all other morphological structure lengths/widths were dependent variables. The segmented model determines the allometric relationship between the dependent and independent variables by fitting two or more straight lines to the data, which are connected at 'breakpoints' where the rate of relative growth changes, as identified by a change in the respective slopes. Segmented regression models were fitted for paired dependent-independent variables, using the package '*segmented'* for R (Muggeo, 2008). This package estimates breakpoints by iteration from an initial value. A set of initial values was built from an interval (mean CL ± 1 standard deviation) in 0.5 mm steps from the lower to the upper extreme of this interval, and the model was run as many times as the number of initial values in this set. A single output was expected for all runs, independent of the initial value at each run, otherwise the analysis was considered inconsistent. In addition, t-values related to the 'gap' (parameter that measures the gap between the two fitted straight lines coming from the model) were recorded, with t-values > 2 indicating problems in model convergence (Muggeo, 2008). The Davies test (Muggeo, 2008) was used to check whether there was a significant difference between the slope parameters of the two straight lines coming from the model. When these two straight lines presented significantly different slopes according to the Davies test, the

MSOM estimate was defined as the value of the independent variable (= CL) associated with the intersection point (breakpoint) between the lines. The coefficient of variation (CV i.e. ratio between the standard deviation and average value of the MSOM estimates) concerning the set of MSOM estimates from different body structures within the FUs were then calculated for each FU / Year group. It was established in this study that a CV higher than 5% indicates high variability.

Additionally, linear regressions were carried out with log transformed data of carapace length (independent variable) and dimensions (length, width or depth) of each body structure (dependent variable) that presented significant breakpoints in the segmented regression. The aim was to check the existence and type of allometry between these body characters and carapace length, according to the equation below:

$$Y = aX^b \Leftrightarrow logY = \log(a) + blog(X) \text{ (eq.1)}$$

Where Y and X represent respectively the dimension of the body structure and the carapace length of any individual in the study, while b and a are respectively the slope and anti-log of the intercept [log (a)] of the regression lines above-mentioned. The relative growth between the body structures and carapace length was classified as follows: (i) isometric growth, when the slope was not significantly different of 1 (b = 1) and (ii) allometric growth, when the slope was significantly different of 1, in this case negative allometric growth, when $0 < b < 1$ and positive allometric growth, when $b > 1$. Significant difference between b and 1 (isometric growth) was assessed by observation of the 95% confidence interval of the regression line's slopes obtained from the log-transformed linear regressions above-mentioned. It is important to highlight that it is expected changes in relative growth of body structures to CL after the MSOM will be consistent with the type of allometry obtained by the log-transformed linear regression, i.e., body structures with positive allometric growth will increase the rate of relative

growth to the CL after reaching the MSOM, while the opposite is expected for body structures with negative allometric growth. Concerning structures with isometric growth, it is expected that the segmented regression analysis will not determine any breakpoint (MSOM).

4.2.3.2. Discrimination analysis of *Nephrops* morphometrics across FUs

Canonical discriminant analysis (CDA) was carried out to investigate the existence of significant differences in the morphometric structure of males and females (separately) among the grounds: Moray Firth (FU9), Firth of Clyde (FU13), Western Irish Sea (FU15), Aran Grounds (FU17) and The Smalls (FU22). Additionally, classificatory discrimination analysis was carried out to examine the classification success of males and females (separately) in the fishing grounds above-mentioned. Only samples obtained in 2018 were considered in the discrimination analysis since all morphometric measurements were made with the same instrument (digital callipers, see above) with identical precision. Therefore, samples obtained at FU17 and FU22 in 2017 that were measured with analogue vernier callipers were omitted from the discriminant analysis (although these were included in MSOM estimation – see above). In this study, 'morphometric structure' refers to the set of scores (output of the CDA) obtained from the morphometric measurements of body structures, which characterize *Nephrops* samples at each FU considered in the study. The morphometric structures were considered distinct whether they presented significantly different centroids (non-overlapping confidence intervals).

Before the CDA, the measurements were standardized for body size, according to a method for correction of size-dependent variation in morphometric characters suggested by Elliot et al. (1995) using Eq. 1 below:

$$M_s = M_o(L_s/L_o)^b \quad \text{(Eq. 2)}$$

Where M_s is the standardized measurement; M_o is the original measurement; L_o is the standard length of the specimen (carapace length); L_s is the overall mean of the standard length for all

lobsters from all the samples in each analysis and *b* is estimated as the slope of the linear regression between $\log(M)$ and $\log(L_o)$ for all lobsters in the analysis. The correlation coefficients between each pair of morphometric characters were checked before and after the removal of the size effect. It was expected that the correlation coefficient between these pairs would decrease after the removal of the size effect (Chen *et al.*, 2015). Only standardized measurements with Pearson correlation coefficient less than 0.70 were included in the discrimination analysis. The CDA and the classificatory analysis were carried out using the R functions described in Koutecký (2014) which are based on the R package *vegan* (Oksanen *et al.*, 2013). There was no necessity to check the samples for normality, since the *vegan* package uses permutation significance tests which overcome the requirement for normal distributions in the morphometric measurements (Koutecký, 2014). While the assumption of similarity of population covariance matrices failed, the analysis was carried out as it is not considered as a required prerequisite for using CDA (Cruz-Castillo *et al.*, 1994). Furthermore, Cocozzelli (2008) emphasizes that CDA can be used with confidence, since there is evidence that it is quite robust to the violation of assumptions of samples normality and homogeneity of covariance matrices.

4.2.3.3. MSOM, morphometric structure and population density

Population densities of *Nephrops* across functional units were obtained from annual underwater television (UWTV) surveys carried out from 2001 to 2018 by the National Institutes in charge of the stock assessment of this species (ICES, 2019a, b). The mean of the annual estimates of population density was calculated for each FU (Table 4.2) and these values used to investigate any pattern between population density across the FUs and MSOM. Significant MSOM estimates considering each body structure separately were compared by least-squares linear regression with the mean of the annual population density estimates (Table 4.2) from each FU / year group. According to Queirós *et al.* (2013), to assure credible

regression models, regressions were only calculated when there were at least 5 significant MSOM estimates for the body structure in question across the FU / year groups.

To investigate any density-dependent pattern among morphometric structures (identified in CDA) across the FUs, significant differences in annual estimates of population density at these FUs were first tested using Kruskal-Wallis and Dunn (post-hoc) tests. After that, the similarity / dissimilarity in morphometric structure of *Nephrops* was tested among FUs with and without significant differences in population density by permutation significance tests included in the *vegan* package.

4.3. Results

4.3.1. Size at the onset of maturity from morphometric data (MSOM)

MSOMs were estimated using pairs of morphological measurements that generated significant breakpoints in segmented regressions, where individual structure dimensions were dependent variables versus CL (independent variable). MSOMs can be seen for females and males, respectively, in Tables 4.3-4.4. For most of the morphometric features examined, particularly in females, MSOM estimation was not possible. These results were recorded as 'NA', for one of the following reasons: (i) the model had not converged (t-values > 2 or some points obstructed the model fitting), (ii) the segmented relationship between the pair of measurements was non-significant, or (iii) there were different outputs from the range of initial values provided to the model. The morphological structure with the highest number of significant MSOM estimates across the FUs for females was 'CL-carapace width' with 4 significant estimates per 7 FU / years analysed.

Several structures gave significant MSOM estimates in males, including 'CL-appendix masculina' and 'CL-1st pleopod', with significant estimates for all the 7 FU / years analysed in the study. 'CL-Crusher length' (males) also had a good number of significant estimates in 6 out of 7 FU / years. However, except for MSOM estimates of females in The Smalls (FU22) in

2018 and males in Moray Firth (FU9) in 2018 (CV = 3.29 and 0.11%, respectively – see Tables 4.3-4.4), the MSOM estimates generated by different pairs of body structures themselves presented great variability even when these were otherwise consistent in terms of providing a significant breakpoint. For example at FU22 in 2018 (CV = 13.23%), CL-1st pleopod gave an MSOM that was ~9 mm smaller than the MSOM from CL-crusher length at the same FU / Year; while at FU17 in 2017 (CV = 20.88%), variability in MSOM from these structures was even higher (up to 10mm CL, Table 4.4). Neither was there any consistency in particular morphometric structures erring towards providing larger or smaller estimates (Tables 4.3-4.4). As many individuals were damaged, the sample size available for segmented regression was greatly reduced for some body structures like chelipeds (see Tables 4.3-4.4). The following body structures increased their rate of relative growth to the CL after reaching the MSOM: CW, WL2-CL, CutD, CutW and CutL (both sexes). Conversely, AbW, CTel and ETel decreased theirs for both sexes as well. For some structures in males (CruW, WL2-MW, CruL and CruD), there was an increase in the rate of relative growth to CL, while in females, for the same body structures, the opposite was observed. Furthermore, in males, WL2-MW and WL2-CW exhibited either an increase or a decrease in the rate of relative growth to CL at different functional units. It is important highlight that the rate of relative growth of body structures to CL after reaching MSOM was usually inconsistent with the type of allometry of such body structures (see 4.2.3.1 in methods for details), except for the ones displayed in Tables 4.5 and 4.6, respectively, for males and females. Examples of segmented regression plots for the structures which gave significant breakpoints most consistently, as well as inconsistent outputs concerning the rate of relative growth of body structures to CL after MSOM and the type of allometry can be seen respectively in Supplementary Figures A4.1-A4.3 and Tables A4.2-A4.3 in appendix 3.

4.3.2. Discrimination analysis of *Nephrops* morphometrics across FUs

Correlation coefficients between morphometric characters of males and females before and after the removal of size effect are shown in Tables 4.7 and 4.8. The coefficients were extremely high before the correction of size-dependent variation, but these were substantially reduced after standardization of the measurements of the morphometric characters. Some pairs of morphometric structure measurements presented correlation coefficients higher or equal to 0.70 and, thus, measurements of only one structure in these pairs were considered in the analysis. The highly correlated pairs were BL-ETel, BL-CTel and ETel-CTel for males (Table 4.7, with ETel and Ctel removed from the analysis) and CTel-AbL, CruW-CruD and CutW-CutD for females (Table 4.8, with CTel, CruW and CutW removed from the analysis). Table 4.9 shows summarized results for the canonical discrimination analysis carried out to investigate differences in the morphometric structure of male and female *Nephrops* per FU. The analysis for both males and females presented 2 significant canonical axes or eigenvectors (Table 4.9), which together explained 89.5 and 89.0% of the variation in the overall morphometric structure respectively for males and females among FUs. Scatterplots of the scores from 3 canonical axes (CA1, CA2 and CA3) for morphometric characters of male and female *Nephrops* respectively are shown in Figures 4.2 and 4.4. The analysis could easily discriminate morphometric structures of males among the grounds with a high degree of separation between Western Irish Sea (FU15), Aran Grounds (FU17) and the group formed by the following FUs: Moray Firth (FU9), Firth of Clyde (FU13) and The Smalls (FU22), which presented a high degree of overlap. There was a certain degree of overlap in the morphometric structure of females at all FUs (Figure 4.4), however the plot of the morphometric structure centroids with their respective 95% confidence intervals (Figure 4.5) shows three distinct groups: (i) Aran Grounds (FU17); (ii) Moray Firth (FU9) and The Smalls (FU22) and (iii) Firth of Clyde (FU13) and Western Irish Sea (FU15). The following structures contributed

significantly ($p < 0.05$) to the discrimination of the samples: (i) BL, CW, AbW, CruL, CutL, CutD, WL2-ML, WL2-CL, WL2-CW, AM and Pl1, for males and (ii) BL, ETel, AbL, WL2-ML, WL2-MW, WL2-CL and WL2-CW, for females (see Table 4.10). Obviously, the first canonical axis (CA1) was the most important component contributing to separation among populations. For males, these differences were exclusively due to shape changes related to the length of the first pleopod and appendix masculina (see Table 4.10 and Figure 4.2), while for females, they were primarily due to shape changes related to the length and width of the second walking leg structures (WL2-ML,WL2-CL and WL2-MW) and to longitudinal body shape changes, such as abdomen, eye-telson and body lengths (AbL, ETel and BL, see Table 4.10 and Figure 4.4). Concerning the second canonical axis (CA2), differences among female populations were associated to morphological changes related to the same structures contributing to the discrimination in the first canonical axis plus WL2-CW (see Table 4.10 and Figure 4.4). For males, the differences in this canonical axis (CA2) were due to shape changes related to the length and width of the second walking leg structures (WL2-ML,WL2-CL and WL2-CW), length and depth of chelipeds (CruL, CutL and CutD), as well as longitudinal and transversal body shape changes such as body length (BL) and abdomen and carapace width (AbW and CW), respectively [Table 4.10 and Figure 4.2]. It is important highlight that the measurements of primary importance in the first and second eigenvectors were respectively first pleopod (0.30) and carpus length of the second walking leg (0.47) for males, and carpus length (- 0.58) and width of the second walking leg (0.47) for females (see Table 4.10).

The classification success of the discrimination analysis ranged between 42.87-100% and 0-85.48% individuals correctly classified, respectively, for male and female *Nephrops* across the grounds considered in the study (Tables 4.11-4.12). As expected by observation of a certain degree of overlap of female scores for the grounds discriminated by the CDA (contrasting with less overlap in male scores, see Figures 4.2 and 4.4), the classification success was lower for

female than males. Similarly, lowest classification success was observed in grounds where the morphometric structures of individuals could not be discriminated by the CDA, those grounds include: (i) The Smalls (grouped with Moray Firth and Firth of Clyde) with 42.87% of males correctly classified and (ii) The Smalls (grouped with Moray Firth) and Firth of Clyde (grouped with Western Irish Sea) both with 0% of females correctly classified.

4.3.3. MSOM, morphometric structure and population density

We required at least 5 valid MSOM estimates per body structure (out of a possible 7) to carry out the comparison between MSOM and population density. This requirement was only met for the following body structures of males: crusher propodus length, appendix masculina and first pleopod. However, there was no significant relationship between these structures and population density across the grounds considered in the study (details in Supplementary Tables A4.4-A4.6 in appendix 3). Few valid MSOM estimates were available in females to make a comparison with density.

Kruskal-Wallis / Dunn tests showed significant differences in population density (both males and females) among the FUs which formed three distinct groups: (i) Firth of Clyde (FU13) and Western Irish Sea (FU15) - 'high density group'; (ii) Aran Grounds (FU17) and The Smalls (FU22) - 'moderate density group' and (iii) Moray Firth (FU9) - 'low density group'. The hypothesis of similarity / dissimilarity in morphometric structure of *Nephrops* among grounds with / without significant differences in population density was rejected. The morphometric structure of male *Nephrops* from Firth of Clyde and The Smalls, which were FUs of high and moderate population density respectively, were grouped with *Nephrops* from the Moray Firth, which is characterized by low population density (see Figure 4.3); while the morphometric structure of female *Nephrops* in Moray Firth (low density) was grouped with the one from The Smalls, characterized by moderate density (see Figure 4.5).

4.4. Discussion

The main objective of this study was to determine the size at the onset of maturity using morphometric criteria and to test the hypothesis of an inverse relationship between this metric and population density in the grounds considered in this study. Unfortunately, despite discovering some morphometric features which consistently gave rise to statistically significant allometric breakpoints, particularly in male *Nephrops*, most of the morphometric-based maturity 'MSOM' results showed great variability within FU / year groups (CV > 5%, see Tables 4.3 and 4.4). For this reason, it was difficult to choose which morphometric measure was the most representative, as was also noted in previous studies (Tuck *et al.*, 2000; McQuaid *et al.*, 2006; Queirós *et al.* 2013). This alone is a major issue, but there may be others, as for example, inconsistency between the type of allometry of body characters and the behaviour of their rate of relative growth to CL after MSOM or the irregular pattern of body structures' allometry and the behaviour of the rate of relative growth to CL after MSOM, with the same body structure presenting contrasting types of allometry (positive or negative) and behaviour of the rate of relative growth (increasing or decreasing after MSOM) at different FUs. Observing the existence of different type of allometric relationships, e. g., sigmoidal allometry (e.g. Rasmussen and Tan, 1992; Nešetřilová, 2005), perhaps, log-transformed and segmented regression models are not appropriate to describe the actual type of allometric relationships in *Nephrops*. Indeed, Katsanevakis *et al.* (2007) pointed out that these models are not adequate to describe allometric relationships of some marine species. They suggest, for example, that quadratic and cubic models are the best models to describe allometric relationships in two species of decapod crustaceans (such as *Nephrops*): sand ghost shrimp (*Pestarella tyrrhena*) and marbled rock crab (*Pachygrapsus marmoratus*), respectively. These authors state that "the allometric exponent (b) in eq. 1 is not necessarily constant and it may change either continuously or abruptly at specific breakpoints" and consequently log-transformed and

segmented regression analysis may confound positive with negative allometry or isometric with allometric growth and vice versa. Wynne (2016) suggests, for example, that, because allometric growth takes place only during a certain period of the life cycle, segmented regression analysis should identify two breakpoints (not one) related to the beginning and end of the period of allometric growth (see Conan *et al*. 2001 for other reservations with using this method). Observing what was discussed above, the variability in MSOM estimates, including variable allometric growth relationships (positive or negative) and variability of MSOM estimates, may be due to the inability of the method to identify the beginning and end of the period of allometric growth or even to estimate points truly related to *Nephrops'* maturity. However, further investigations might clarify these issues.

Supposing log-transformed and segmented regression analysis are adequate to describe allometric relationships in *Nephrops*, the discussion will continue highlighting that despite extremely small sample sizes (n = 10), crusher dimensions (length, depth and width) of females provided significant breakpoints, with SOM estimates in the range 22.26-23.71 mm CL at FU22 in 2018. These results contradict the assertion that large sample sizes are essential for identifying significant segmented relationship in morphometric analysis (Queirós et al., 2013), although they might be lucky accidents. Most of the MSOM structures showed an irregular pattern, providing significant outputs for only some of the FUs analysed in the study. Finally, it was difficult to decide based on MSOM estimates, which structure is the best for morphometric SOM estimation. Perhaps the appendix masculina and the male first pleopod (considered for the first time in this study) are good structures for males, since they provided significant estimates for all the FU / years studied. Additionally, these structures (appendix masculina and male first pleopod) provided consistent results at 25.09 and 25.63 mmCL, respectively, with previous studies in Irish Sea by McQuaid et al. (2005, 2006) that reported MSOM estimates obtained from appendix masculina in the range 24.3-26.9 mmCL across a

variety of stations at this ground. The MSOM estimate obtained from appendix masculina for Firth of Clyde in this study was also consistent with the one obtained by Queirós *et al.* (2013) for the same site and body structure (both ~27 mmCL), although the result obtained from the male first pleopod in the present study was a little bit higher (29.96 mmCL) for the same site. It is important to emphasize that there is a relational order between the different maturity metrics with size at the onset of maturity preceding the size at which 50% of the sample is mature (L_{50}) and smallest berried female, as discussed in da Silva Santana *et al.* (2021). In that respect, the significant MSOM measures identified in the present study remain difficult to interpret. We note, for example, that whatever body structure used, MSOM estimates for females in this study fluctuate in an irregular pattern around the L_{50} estimates reported in previous studies for Moray Firth, Firth of Clyde, Irish Sea, Aran Grounds and The Smalls (de Figueiredo and Thomas, 1967; Tuck *et al.*, 2000; McQuaid *et al.*, 2006; da Silva Santana *et al.*, 2021). In other words, the same body structure provides MSOM estimates that are higher than L_{50} for some grounds and lower for others.

There was no significant inverse relationship between MSOM and population density, contrasting with Queirós et al. (2013), who reported two significant inverse relationships between MSOM (from crusher length / appendix masculina) and population density for males in Scottish waters. Similarly, da Silva Santana *et al.* (2021) showed that there was an inverse relationship between the theoretical size at the onset of maturity (=TSOM) in females and population density in Irish waters. Possibly, the absence of a significant relationship herein is due to the low number of points in the regression analysis (5-7 depending on the body structure), since Jenkins and Quintana-Ascencio (2020) state that regressions with sample size less than 25 ($n < 25$) may be inaccurate or unstable.

Secondary objectives of this study were to show variation in morphometric structure in *Nephrops* populations from Irish and Scottish FUs and to test the hypothesis that such

variations were related to a gradient in population density values across these sites. Indeed, significant differentiation in morphometric structure was evident on different grounds, which was somewhat surprising, given the openness of these populations from a genetic viewpoint. Recent studies have shown a substantial gene flow among these stocks (Maltagliati *et al.*, 1998; Passamonti *et al.*, 1997; Stamatis *et al.*, 2004; Streiff *et al.*, 2001), probably as a result of highly-dispersing larvae (McGeady *et al.*, 2020). The canonical discriminant analysis could discriminate male and female *Nephrops* across the sites considered in the study.

However, the hypothesis of a link between morphometric structure and density across the FUs was rejected since morphometric structure of females in The Smalls and of males in The Smalls and Firth of Clyde, sites of moderate (Smalls) and high (Firth of Clyde) population density, were not significantly different from those of females and males in Moray Firth (FU9), a functional unit of low population density. Maynou and Sardà (1997) have shown a link between the morphometric structure of *Nephrops* populations at different sites in Mediterranean Sea and the type of sediment on these grounds. Since there is a dome shaped relationship between the sediment type (silt+clay content) and population density (Campbell *et al*., 2009; Johnson *et al*., 2013), it was expected the hypothesis linking morphometric structure and population density would be accepted.

It is important emphasize that, despite the fact that the hypothesis linking density and morphometric structure has been formally rejected, we believe that population density still has a role in shaping morphometrical structure of *Nephrops* populations, since the CDA could discriminate the morphometric structure of *Nephrops* from sites of contrasting densities to some extent, except for Firth of Clyde and The Smalls (see Figures 4.3 and 4.5), however Firth of Clyde has a known density-size gradients operating within it. Note that this discrimination based on morphometric structure is independent of body size since this was standardized prior to statistical analysis.

Sediment type (granulometry specifically the silt+clay content), which influences *Nephrops* density (Campbell *et al*., 2009; Johnson *et al*., 2010) probably indirectly affects morphometrics *via* density effects on morphometrics. Other environmental conditions among the studied sites such as differences in alkalinity, current pattern, temperatures, turbidity, and environmental impacts could also affect morphometry (Marr, 1957; Maynou and Sardà, 1997; Siddik et al., 2016), or (more likely?) these could co-vary with sediment type. Another factor may be habitat usage; Vermeiren *et al.* (2020) showed a close relationship between morphometrical traits in crab species, such as differences in the eyestalks length and position in the carapace and distinct habitats with differing necessity of telescopic or stereoscopic view. Other studies have suggested a functional link between habitat usage / characteristics and morphological traits in different aquatic organisms, e. g. claws of portunid crabs of different trophic niches (Freire *et al.*, 1996), carapaces of benthic and pelagic marine shrimps (Duarte *et al.*, 2016), legs of benthic and swimming crabs (Marochi and Masunari, 2016) and locomotion appendages of amphipods (Kralj-Fišer *et al.*, 2020). Vermeiren *et al.* (2020) highlight that various factors have some influence on the morphology of organisms (e.g. sexual variation, allometric relations, diet, phylogeny) and suggest an integrative and multi-factor approach in future studies. Please note, that all the studies mentioned above compared morphometric traits across species, by contrast with the present study, that has compared these traits within one species. Future studies may indicate the extent of influence which habitat usage has on the morphological structures of *Nephrops*, particularly the second walking leg, that is extremely active in some ecological and social tasks (see below).

Gomes *et al.* (2016) emphasize that morphological traits are linked to the environment and act to maximize the ability of an individual to accomplish ecological and social tasks, assure survivorship and improve reproductive capacity. Indeed, the most important body structures for the discrimination of *Nephrops* populations across the FUs in this study were the first

pleopod (males) and second walking leg (males and females, Table 4.10). These two body structures (first pleopod and second walking leg) are actively used by male *Nephrops* during copulation (see Farmer, 1974c). The second pair of walking legs is also actively used by both male and female for digging burrows (Maynou and Sardà, 1997), which are very important means of providing a protected shelter against predation and antagonistic interactions, including the lengthy embryo brooding period for females. Perhaps distinct walking leg shapes are related to the degree of difficulty in excavating the burrows on different types of sediment across the FUs, which could induce growth and development of walking legs of slightly different shapes, but only further investigation might clarify this hypothesis. The lack of genetic structure means that this must be an ecological difference that is stimulated by the environment rather than one that is determined genetically.

Besides the male first pleopod and second walking leg, other body structures contributed significantly to the discrimination of *Nephrops* across the FUS: appendix masculina and claw variables (CutL, CutD and CruL) in males, and abdomen (AbL) and claw (CruD) variables in females. Appendix masculina and female abdomen are related to the reproductive process in *Nephrops*, a functional appendix masculina is essential for a successful copulation (Haig *et al.*, 2016; Waiho *et al.*, 2017), while a longer and wider abdomen enhances the capacity for egg/embryo carrying (Farmer, 1974a; McQuaid *et al.*, 2006; Queirós *et al.*, 2013; Haig *et al.*, 2016; Waiho *et al.*, 2017). The claws are related to sexual selection of males (by females), foraging, as well as as fighting for burrows and sexual mates (Sbragaglia *et al.*, 2017), which may be intense, especially in sites of high population density that host more aggressive males (Merder *et al.*, 2019).

This study has shown that *Nephrops* from the Irish functional units (Aran Grounds, The Smalls and Western Irish Sea) in this study can be considered distinct regarding their morphometric structure (Figures 4.2-4.5). Surprisingly, despite the fact that the male and female samples from

The Smalls were discriminated from the other Irish FUs, samples from this ground could not be separated from the Scottish FUs at all, regardless of the large geographic distance separating these grounds. Further investigations might explain which factors are associated with the similarity in morphometric structure of samples from these grounds. Concerning the Scottish samples (Moray Firth and Firth of Clyde), only female samples could be discriminated according to their morphometric structure, however, it is important emphasize that the centroids of the female morphometric scores of these two FUs were very close one to the other as indicated by the almost overlapping 95% confidence intervals (Figure 4.5), what indicate a low degree of separation between them.

The percentage of correctly-classified individuals supports the output of the CDA with three well separated groups of males with no overlapping morphometric structure: (i) Western Irish Sea (100% individuals correctly-classified), (ii) Aran Gorounds (100% individuals correctly-classified) and (iii) the cluster Moray Firth-Firth of Clyde-The Smalls with 68, 69.57 and 42.86% individuals correctly-classified, respectively. Naturally, the lower percentage in the classification of individuals at Moray Firth, Firth of Clyde and The Smalls when compared to the other FUs reflects the existence of overlapping morphometric structures among them. Concerning females, the percentage of success in the classification was lower when compared to males', corroborating with overlapping morphometric structures for the functional units in the study, with a high degree of overlap between some of them. The percentage of individuals correctly-classified was: (i) 52.94% (Moray Firth), (ii) Firth of Clyde (0%), (iii) 75.61% (Western Irish Sea), (iv) 85.48% (Aran Grounds) and (v) The Smalls (0%).

Tzeng *et al.* (2001) state that the following factors may confound the morphometric relationship in a population: (i) sexual dimorphism; (ii) time of sampling; (iii) allometric growth and (iv) state of maturity. We believe that the first three factors above were not an issue in the present analysis, since it was carried out separately for males and females, all the samples

were collected in the summer of 2018 and all measurements were corrected for the effect of body size. To account for the final factor 'state of maturity', it would have been necessary to restrict group comparisons to specific length classes. However, maturity was not an issue in other studies on variation in the morphometric structure of decapods, such as the red-spot prawn (Tzeng *et al.*, 2001) and the southern pink shrimp (Paramo and Saint-Paul, 2010), so arguably, the same may be true for *Nephrops*.

This study showed, for the first time, variation in morphometric structure in *Nephrops* populations in the northeast Atlantic and presented canonical discrimination analysis as a potential tool to separate these populations. In addition, it showed that population density seems to be an important factor influencing the morphometric structure of some populations of this species, as well as highlighting structures (male first pleopod and second walking leg) of primary importance in discriminating these populations. Finally, it presented the male first pleopod as a potential body structure for determination of the onset of maturity estimation using morphometrics. It is expected that the findings of this investigation might contribute to the management of *Nephrops* stocks, observing 'The Marine Strategy Framework Directive and reformed Common Fisheries Policy (CFP)' that require ecosystem-based fisheries management that observes interactions among all components of the ecosystem.

4.5. Tables

Table 4.1. Morphometric measurements used for morphological size at onset of maturity (i.e. 'MSOM') estimation in previous studies in *Nephrops*, including sex-specific differences. Please see Figure 1 for further details on body structures and measurement dimensions

Measurement	Abbreviation	Sex	Previous studies	Dimensions
Abdomen length	AbL	Male / Female	McQuaid *et al.* (2006)	Fig. 1(a)
Abdomen width	AbW	Male / Female	Tuck *et al.* (2000)	Fig. 1(a)
Appendix masculina	AM	Male	Farmer (1974a)	Fig. 1(b)
Body length	BL	Male / Female	Mori *et al.* (1996)	Fig. 1(a)
Carapace length	CL	Male / Female	Maynou and Sardà (1997)	Fig. 1(a)
Carapace width	CW	Male / Female	Maynou and Sardà (1997)	Fig. 1(a)
Carapace-telson length	CTel	Male / Female	Mori et al. (1996)	Fig. 1(a)
Crusher propodus depth	CruD	Male / Female	Queirós *et al.*, 2013	Fig. 1(c)
Crusher propodus length	CruL	Male / Female	Queirós *et al.*, 2013	Fig. 1(a)
Crusher propodus width	CruW	Male / Female	Queirós *et al.*, 2013	Fig. 1(c)
Cutter propodus depth	CutD	Male / Female	Queirós et al., 2013	Fig. 1(c)
Cutter propodus length	CutL	Male / Female	Queirós *et al.*, 2013	Fig. 1(a)
Cutter propodus width	CutW	Male / Female	Queirós *et al.*, 2013	Fig. 1(c)
Eye-telson length	ETel	Male / Female	Mori *et al.* (1996)	Fig. 1(a)
1st pleopod	Pl1	Male	This study	Fig. 1(b)
2nd Walking leg Merus Length	WL2-ML	Male / Female	Maynou and Sardà (1997)	Fig. 1(a)
2nd Walking leg Merus Width	WL2-MW	Male / Female	Maynou and Sardà (1997)	Fig. 1(a)
2nd Walking leg Carpus Length	WL2-CL	Male / Female	Maynou and Sardà (1997)	Fig. 1(a)
2nd Walking leg Carpus Width	WL2-CW	Male / Female	Maynou and Sardà (1997)	Fig. 1(a)

Table 4.2. Annual population density (burrows m^{-2}) obtained from annual underwater television (UWTV) surveys carried out across Irish and Scottish functional units: Moray Firth (FU9), Firth of Clyde (FU13), Western Irish Sea (FU15), Aran Grounds (FU17), and The Smalls (FU22) (ICES, 2019a, b).

Year	FU9	FU13	FU15	FU17	FU22
2001	0.16	0.71	-	-	-
2002	0.24	0.76	-	0.79	-
2003	0.33	0.87	0.99	0.94	-
2004	0.29	0.95	1.00	1.08	-
2005	0.40	0.94	1.02	0.81	-
2006	0.21	0.88	0.97	0.46	0.49
2007	0.24	0.60	0.93	0.69	0.37
2008	0.21	0.85	0.77	0.41	0.36
2009	0.19	0.72	0.83	0.52	0.36
2010	0.18	0.84	0.90	0.63	0.37
2011	0.17	1.04	0.88	0.51	0.41
2012	0.14	0.68	0.91	0.33	0.49
2013	0.21	0.96	0.78	0.33	0.41
2014	0.15	0.64	0.83	0.28	0.53
2015	0.16	0.88	0.79	0.40	0.49
2016	0.18	0.94	0.84	0.29	0.31
2017	0.19	0.75	0.90	0.31	0.55
2018	0.19	1.06	0.85	0.40	0.31
Mean (burrows m^{-2})	0.21	0.84	0.89	0.54	0.42

Table 4.3. Size at the onset of maturity of female *Nephrops* estimated from morphometric data (i.e. 'MSOM', CL mm): Moray Firth (FU9), Firth of Clyde (FU13), Western Irish Sea (FU15), Aran Grounds (FU17) and The Smalls (FU22) and coefficient of variation of MSOM estimates obtained from different body structures at each functional unit. NA indicates no significant allometric breakpoint.

Structure	Size at the onset of maturity of female *Nephrops* from morphometric data – MSOM (mm)						
	FU9	FU13	FU15	FU17		FU22	
	2018	2018	2018	2017	2018	2017	2018
Abdomen length	NA	NA	**21.81** (n=247)	**25.29** (n=238)	**26.03** (n=250)	NA	NA
Abdomen width	NA	NA	NA	NA	NA	**32.25** (n=230)	NA
Body length	NA	NA	**21.89** (n=220)	NA	NA	NA	NA
Carapace width	NA	NA	**21.57** (n=250)	**17.84** (n=239)	**27.06** (n=250)	**31.42** (n=229)	NA
Carapace-telson length	**25.46** (n=50)	NA	**23.56** (n=247)	NA	NA	NA	NA
Crusher length	NA	NA	NA	NA	NA	NA	**22.26** (n=10)
Crusher width	NA	NA	NA	NA	NA	NA	**22.64** (n=10)
Crusher depth	NA	NA	NA	NA	NA	NA	**23.71** (n=10)
Cutter length	NA	NA	NA	NA	NA	NA	NA
Cutter width	**37.18** (n=30)	NA	NA	NA	NA	NA	NA
Cutter depth	**37.17** (n=30)	NA	NA	**34.83** (n=95)	NA	NA	NA
Eye-telson length	NA	NA	**22.07** (n=247)	NA	**21.70** (n=250)	NA	NA
2nd W. L. merus length	NA	**19.69** (n=24)	NA	NA	NA	NA	NA
2nd W. L. merus width	NA	NA	NA	NA	NA	NA	NA
2nd W. L. carpus length	NA	NA	**33.40** (n=183)	NA	**22.54** (n=192)	NA	NA
2nd W. L. carpus width	NA	NA	NA	NA	NA	**20.10** (n=187)	NA
CV (%)	20.33	NA	19.27	32.77	10.73	24.31	3.29

Table 4.4. Size at the onset of maturity of male *Nephrops* estimated from morphometric data (i.e. 'MSOM', CL mm): Moray Firth (FU9), Firth of Clyde (FU13), Western Irish Sea (FU15), Aran Grounds (FU17) and The Smalls (FU22) and coefficient of variation of MSOM estimates obtained from different body structures at each functional unit. NA indicates no significant allometric breakpoint.

Structure	Size at the onset of maturity of male *Nephrops* from morphometric data – SOM (mm)						
	FU9	FU13	FU15	FU17		FU22	
	2018	2018	2018	2017	2018	2017	2018
Abdomen length	NA	NA	**33.29** (n=250)	NA	**30.40** (n=249)	**27.72** (n=247)	NA
Abdomen width	**35.60** (n=45)	**35.04** (n=65)	NA	NA	NA	NA	NA
Appendix masculina	**35.68** (n=44)	**26.68** (n=43)	**25.09** (248)	**20.21** (n=129)	**22.46** (n=105)	**27.00** (n=42)	**27.07** (n=158)
Body length	NA	NA	NA	NA	NA	NA	NA
Carapace width	NA	NA	**32.88** (n=250)	**24.79** (n=217)	NA	NA	NA
Carapace-telson length	NA	NA	**26.60** (n=249)	NA	NA	NA	NA
Crusher length	NA	**27.97** (n=51)	**26.91** (n=137)	**29.90** (n=54)	**25.54** (n=98)	**29.89** (n=115)	**36.39** (n=30)
Crusher width	NA	**23.50** (n=51)	NA	NA	NA	NA	NA
Crusher depth	NA	NA	**29.27** (n=137)	NA	NA	NA	NA
Cutter length	NA	**31.99** (n=49)	**25.50** (n=80)	NA	**26.00** (n=114)	NA	NA
Cutter width	NA	NA	**26.20** (n=80)	NA	**29.88** (n=114)	NA	**33.37** (n=35)
Cutter depth	NA	NA	**26.24** (n=80)	NA	NA	NA	NA
Eye-telson length	NA	NA	**26.78** (n=249)	NA	**24.29** (n=249)	**27.64** (n=247)	NA
1st pleopod	**35.63** (n=44)	**29.96** (n=58)	**25.63** (n=249)	**19.66** (n=129)	**22.43** (n=231)	**25.10** (n=42)	**27.32** (n=158)
2nd W. L. merus length	NA	NA	**33.12** (n=196)	**30.70** (n=153)	NA	NA	NA
2nd W. L. merus width	NA	NA	**33.04** (n=196)	NA	NA	**29.34** (n=211)	**29.32** (n=107)
2nd W. L. carpus length	NA	NA	**26.54** (n=195)	**32.16** (n=152)	NA	NA	NA
2nd W. L. carpus width	NA	**31.24** (n=62)	**23.24** (n=194)	NA	NA	NA	NA
CV (%)	0.11	12.87	12.11	20.88	12.50	6.17	13.23

Table 4.5. Output of the log-transformed regression model between dimensions of body structures (dependent variable) that provided significant MSOM and consistent output concerning type of allometry and relative growth of the body structure to CL after MSOM versus carapace length (independent variable) of male *Nephrops* from Moray Firth (FU9), Firth of Clyde (FU13), Western Irish Sea (FU15), Aran Grounds (FU17) and The Smalls (FU22), as well as MSOM and behaviour of relative growth of body structures dimensions to CL after MSOM..

Ground	Year	Trait	N	r^2	Intercept	Slope			Diagnosis	MSOM	Relative growth after MSOM
						b	2.5% CI	97.5% CI			
FU9	2018	Pl1	43	0.94	-1.37	0.87	0.80	0.94	Negative allometry	35.63	decrease growth rate
FU13	2018	CruL	51	0.97	-0.48	1.28	1.21	1.34	Positive allometry	27.97	increase growth rate
FU13	2018	CruW	51	0.95	-2.22	1.34	1.26	1.43	Positive allometry	23.50	increase growth rate
FU13	2018	CutL	63	0.93	-0.50	1.28	1.19	1.38	Positive allometry	31.99	increase growth rate
FU13	2018	WL2_CW	62	0.94	-3.17	1.17	1.09	1.24	Positive allometry	31.24	increase growth rate
FU13	2018	Pl1	57	0.74	-0.85	0.72	0.61	0.84	Negative allometry	29.96	decrease growth rate
FU15	2018	CW	242	0.99	-0.87	1.06	1.05	1.07	Positive allometry	32.88	increase growth rate
FU15	2018	CruL	137	0.88	-0.70	1.34	1.26	1.42	Positive allometry	26.91	increase growth rate
FU15	2018	CruD	139	0.89	-2.50	1.33	1.25	1.41	Positive allometry	29.27	increase growth rate
FU15	2018	CutL	115	0.96	-0.40	1.25	1.20	1.30	Positive allometry	25.50	increase growth rate
FU15	2018	CutW	120	0.96	-2.25	1.31	1.26	1.35	Positive allometry	26.20	increase growth rate
FU15	2018	CutD	120	0.88	-2.79	1.37	1.28	1.46	Positive allometry	26.24	increase growth rate
FU15	2018	WL2-ML	196	0.95	-0.64	1.04	1.01	1.08	Positive allometry	33.12	increase growth rate
FU15	2018	WL2-MW	196	0.96	-3.38	1.22	1.19	1.26	Positive allometry	33.04	increase growth rate
FU15	2018	WL2-CL	195	0.95	-1.61	1.07	1.03	1.10	Positive allometry	26.54	increase growth rate
FU15	2018	WL2-CW	194	0.95	-3.07	1.13	1.10	1.17	Positive allometry	23.24	increase growth rate
FU17	2017	CW	217	0.97	-0.86	1.05	1.03	1.08	Positive allometry	24.79	increase growth rate
FU17	2017	CruL	54	0.97	-0.09	1.15	1.10	1.21	Positive allometry	29.9	increase growth rate
FU17	2017	WL2_CL	152	0.90	-1.65	1.07	1.01	1.13	Positive allometry	32.16	increase growth rate
FU17	2018	ETel	249	0.88	1.40	0.91	0.87	0.95	Negative allometry	24.29	decrease growth rate
FU22	2017	CruL	115	0.96	-0.27	1.21	1.16	1.26	Positive allometry	29.89	increase growth rate
FU22	2018	CruL	29	0.97	-0.34	1.23	1.15	1.31	Positive allometry	36.39	increase growth rate
FU22	2018	CutW	35	0.97	-1.88	1.21	1.13	1.28	Positive allometry	33.37	increase growth rate

Table 4.6. Output of the log-transformed regression model between dimensions of body structures (dependent variable) that provided significant MSOM and consistent output concerning type of allometry and relative growth of the body structure to CL after MSOM versus carapace length (independent variable) of female *Nephrops* from Moray Firth (FU9), Firth of Clyde (FU13), Western Irish Sea (FU15), Aran Grounds (FU17) and The Smalls (FU22), as well as MSOM and behaviour of relative growth of body structures dimensions to CL after MSOM..

Ground	Year	Trait	N	r^2	Intercept	Slope			Diagnosis	MSOM	Relative growth after MSOM
						b	2.5% CI	97.5% CI			
FU9	2018	CutD	40	0.90	-2.10	1.13	1.01	1.26	Positive allometry	37.17	increase relative growth
FU15	2018	CW	250	0.95	-1.04	1.10	1.07	1.13	Positive allometry	21.57	increase relative growth
FU17	2017	CW	239	0.97	-0.85	1.05	1.03	1.07	Positive allometry	17.84	increase relative growth
FU17	2018	ETel	250	0.97	1.19	0.98	0.95	1.00	Negative allometry	21.7	decrease relative growth
FU22	2017	CW	230	0.98	-0.93	1.08	1.06	1.10	Positive allometry	31.42	increase relative growth
FU22	2017	WL2_CW	230	0.88	-3.01	1.11	1.05	1.17	Positive allometry	20.1	increase relative growth

Table 4.7. Correlation coefficients between pairs of morphometric characters of male *Nephrops* before (lower diagonal matrix) and after (upper diagonal matrix) the removal of size effect. One structure was removed from the analysis in all cases where it was one of a pair with correlation coefficients higher or equal to 0.70 after size standardization (i.e. in ETel and CTel correlations, indicated in bold).

Variable		BL	CW	ETel	CTel	AbL	AbW	CruL	CruW	CruD	CutL	CutW	CutD	WL2 ML	WL2 MW	WL2 CL	WL2 CW	AM	Pl1
	BL		*0.58*	***0.91***	***0.82***	*0.60*	*0.33*	*0.54*	*0.47*	*0.24*	*0.33*	*0.37*	*0.26*	*0.25*	*0.31*	*0.02*	*0.21*	*0.05*	*0.28*
	CW	0.99		*0.60*	*0.62*	*0.44*	*0.26*	*0.54*	*0.51*	*0.30*	*0.37*	*0.35*	*0.20*	*0.30*	*0.25*	*0.27*	*0.23*	*0.11*	*-0.03*
	ETel	1.00	0.99		***0.91***	*0.64*	*0.34*	*0.53*	*0.48*	*0.26*	*0.36*	*0.39*	*0.26*	*0.27*	*0.35*	*0.11*	*0.21*	*0.12*	*0.29*
	CTel	0.99	0.99	1.00		*0.67*	*0.34*	*0.58*	*0.53*	*0.28*	*0.44*	*0.43*	*0.27*	*0.38*	*0.36*	*0.30*	*0.27*	*0.16*	*0.22*
	AbL	0.99	0.98	0.99	0.99		*-0.18*	*0.44*	*0.38*	*0.20*	*0.35*	*0.33*	*0.23*	*0.23*	*0.25*	*0.17*	*0.22*	*0.11*	*0.06*
	AbW	0.93	0.93	0.93	0.94	0.89		*0.48*	*0.46*	*0.24*	*0.31*	*0.38*	*0.25*	*0.32*	*0.44*	*0.28*	*0.37*	*0.04*	*0.11*
	CruL	0.96	0.97	0.96	0.97	0.96	0.96		*0.61*	*-0.06*	*0.60*	*0.45*	*0.43*	*0.54*	*0.13*	*0.45*	*0.19*	*0.10*	*0.01*
	CruW	0.95	0.96	0.96	0.96	0.95	0.95	0.96		*0.37*	*0.52*	*0.10*	*0.32*	*0.38*	*0.15*	*0.29*	*0.17*	*0.05*	*0.07*
	CruD	0.93	0.94	0.94	0.94	0.93	0.93	0.93	0.97		*0.31*	*0.10*	*0.25*	*0.33*	*0.15*	*0.20*	*0.23*	*0.09*	*-0.05*
	CutL	0.96	0.96	0.96	0.96	0.96	0.96	0.98	0.96	0.93		*0.59*	*0.25*	*0.48*	*0.15*	*0.40*	*0.18*	*0.12*	*-0.06*
	CutW	0.95	0.96	0.96	0.96	0.95	0.96	0.96	0.93	0.91	0.96		*0.38*	*0.32*	*0.30*	*0.25*	*0.28*	*0.04*	*0.07*
	CutD	0.91	0.91	0.91	0.91	0.90	0.91	0.95	0.94	0.92	0.90	0.93		*0.16*	*0.11*	*0.13*	*0.10*	*-0.04*	*0.11*
WL2	ML	0.95	0.95	0.95	0.96	0.95	0.95	0.96	0.94	0.93	0.95	0.94	0.88		*0.15*	*0.44*	*0.17*	*0.04*	*0.06*
WL2	MW	0.94	0.94	0.94	0.94	0.94	0.95	0.91	0.91	0.91	0.91	0.92	0.85	0.91		*0.07*	*0.44*	*-0.15*	*0.16*
WL2	CL	0.89	0.91	0.90	0.91	0.90	0.91	0.92	0.90	0.89	0.91	0.90	0.84	0.92	0.86		*0.11*	*0.12*	*-0.10*
WL2	CW	0.92	0.93	0.92	0.93	0.92	0.93	0.90	0.91	0.91	0.90	0.90	0.84	0.90	0.92	0.86		*-0.09*	*0.05*
	AM	0.83	0.84	0.84	0.84	0.83	0.78	0.79	0.77	0.75	0.78	0.76	0.70	0.79	0.76	0.79	0.76		*-0.17*
	Pl1	0.80	0.77	0.81	0.80	0.78	0.75	0.73	0.74	0.71	0.73	0.74	0.71	0.73	0.74	0.66	0.73	0.50	

Table 4.8. Correlation coefficients between pairs of morphometric characters of female *Nephrops* before (lower diagonal matrix) and after (upper diagonal matrix) the removal of size effect. One structure was removed from the analysis in all cases where it was one of a pair with correlation coefficients higher or equal to 0.70 after size standardization (i.e. in CTel, CruW and CutW correlations, indicated in bold).

Variable		BL	CW	ETel	CTel	AbL	AbW	CruL	CruW	CruD	CutL	CutW	CutD	WL2 ML	WL2 MW	WL2 CL	WL2 CW
	BL		*0.12*	*0.51*	*0.39*	*0.36*	*0.16*	*0.08*	*0.01*	*0.04*	*0.18*	*0.12*	*0.20*	*0.10*	*0.16*	*-0.20*	*-0.04*
	CW	0.86		*0.22*	*0.19*	*0.11*	*0.07*	*0.17*	*0.29*	*0.27*	*0.05*	*0.03*	*0.10*	*0.04*	*0.16*	*0.03*	*0.15*
	ETel	0.97	0.89		***0.84***	*0.65*	*0.26*	*0.17*	*0.20*	*0.19*	*0.26*	*0.10*	*0.21*	*0.10*	*0.18*	*-0.06*	*0.04*
	CTel	0.97	0.89	1.00		***0.70***	*0.23*	*0.23*	*0.30*	*0.21*	*0.35*	*0.08*	*0.23*	*0.20*	*0.19*	*0.18*	*0.18*
	AbL	0.97	0.88	0.99	0.99		*0.25*	*0.28*	*0.36*	*0.25*	*0.31*	*0.11*	*0.27*	*0.23*	*0.17*	*0.08*	*0.21*
	AbW	0.88	0.81	0.91	0.91	0.91		*0.02*	*-0.03*	*-0.02*	*-0.17*	*0.61*	*0.14*	*0.05*	*0.05*	*-0.01*	*0.03*
	CruL	0.97	0.97	0.97	0.98	0.98	0.79		*0.43*	*0.38*	*0.45*	*0.04*	*0.22*	*0.32*	*0.13*	*0.18*	*0.18*
	CruW	0.94	0.95	0.95	0.95	0.95	0.76	0.95		***0.76***	*0.41*	*-0.06*	*0.25*	*0.17*	*0.30*	*0.00*	*0.21*
	CruD	0.92	0.93	0.92	0.92	0.93	0.74	0.93	0.97		*0.35*	*-0.04*	*0.31*	*0.15*	*0.21*	*0.01*	*0.14*
	CutL	0.89	0.75	0.90	0.91	0.90	0.71	0.96	0.94	0.92		*-0.55*	*-0.19*	*0.26*	*0.02*	*0.07*	*0.26*
	CutW	0.54	0.46	0.55	0.54	0.55	0.74	0.54	0.50	0.49	0.26		***0.71***	*-0.01*	*0.09*	*0.06*	*-0.04*
	CutD	0.84	0.72	0.84	0.84	0.84	0.75	0.94	0.92	0.91	0.69	0.77		*0.06*	*0.18*	*0.09*	*0.08*
WL2	**ML**	0.93	0.81	0.93	0.93	0.93	0.84	0.95	0.92	0.89	0.88	0.49	0.79		*0.11*	*0.29*	*0.30*
WL2	**MW**	0.92	0.83	0.92	0.92	0.92	0.82	0.91	0.91	0.88	0.83	0.53	0.81	0.87		*-0.03*	*0.24*
WL2	**CL**	0.85	0.76	0.86	0.88	0.86	0.77	0.87	0.83	0.81	0.80	0.50	0.77	0.87	0.80		*0.20*
WL2	**CW**	0.92	0.83	0.92	0.93	0.92	0.83	0.94	0.92	0.90	0.88	0.48	0.79	0.90	0.89	0.85	

Table 4.9. Output for canonical discriminant analysis carried out based on morphometric characters of male and female *Nephrops* in a series of Scottish and Irish functional units: Moray Firth (FU9), Firth of Clyde (FU13), Western Irish Sea (FU15), Aran Grounds (FU17) and The Smalls (FU22). Eigenvalues, proportion explained and cumulative proportion concerning 2 significant canonical axes (CCA1-2) are summarized, as well as the level of significance of the respective axis.

Results	Male		Female	
	CA1	CA2	CA1	CA2
Eigenvalue	0.9558	0.7106	0.6275	0.2733
Proportion explained (%)	51.36	38.18	61.99	27.01
Cumulative proportion	51.36	89.54	61.99	89.00
p-value	0.001	0.001	0.001	0.031

Table 4.10. Coefficients for the significant eigenvectors (CA1 and CA2) provided by the canonical discrimination analysis based on morphometrical measurements applied to *Nephrops* populations across Irish and Scottish functional units. Structures of primary importance for the discrimination of such populations in the first and second eigenvectors body characters contributing significatively to the discrimination (*p-value* < 0.05) are in bold.

Morphometric structure	CA1		CA2		*p-value*	
	Male	Female	Male	Female	Male	Female
Body length	0.03	0.38	-0.31	- 0.25	**0.01**	**0.01**
Carapace width	0.01	0.10	0.13	0.29	**0.04**	0.06
Eye-telson length	NA	0.27	NA	- 0.15	NA	0.01
Carapace-telson length	NA	NA	NA	NA	NA	NA
Abdomen length	-0.02	0.15	0.10	0.46	0.21	**0.01**
Abdomen width	-0.01	0.01	0.14	- 0.10	**0.01**	0.90
Crusher propodus length	-0.05	- 0.14	0.15	0.10	**0.01**	0.12
Crusher propodus width	0.00	NA	0.11	NA	0.08	NA
Crusher propodus depth	0.01	0.13	0.01	0.30	0.42	0.05
Cutter propodus length	-0.06	- 0.02	0.17	0.32	**0.01**	0.17
Cutter propodus width	0.02	NA	0.06	NA	0.49	NA
Cutter propodus depth	0.04	0.06	-0.17	0.35	**0.01**	0.11
2nd Walking leg Merus Length	-0.01	- 0.27	0.27	0.26	**0.01**	**0.01**
2nd Walking leg Merus Width	0.02	0.34	0.04	0.40	0.27	**0.01**
2nd Walking leg Carpus Length	-0.05	**- 0.58**	**0.47**	0.33	**0.01**	**0.01**
2nd Walking leg Carpus Width	0.04	0.02	0.23	**0.47**	**0.01**	**0.03**
Appendix masculina	-0.29	NA	-0.01	NA	**0.01**	NA
First pleopod	**0.30**	NA	-0.03	NA	**0.01**	NA

Table 4.11. Classification success for the discrimination of the morphometric structure of male *Nephrops* in Irish and Scottish fishing grounds: Moray Firth, Firth of Clyde, Western Irish Sea, Aran Grounds and The Smalls. Sample size (n) refers to the number of individuals analysed after the 'NA' cases have been automatically removed during the analysis.

Population	FU9	FU13	FU15	FU17	FU22	Sample size (n)	**Correct (%)**
Moray Firth (FU9)	0	6	0	17	2	25	**68**
Firth of Clyde (FU13)	0	16	0	6	1	23	**69.57**
Irish Sea West (FU15)	0	0	82	0	0	82	**100**
Aran Grounds (FU17)	27	0	0	0	0	27	**100**
The Smalls (FU22)	0	4	0	0	3	7	**42.86**

Table 4.12. Classification success for the discrimination of the morphometric structure of female *Nephrops* in Irish and Scottish fishing grounds: Moray Firth, Firth of Clyde, Western Irish Sea, Aran Grounds and The Smalls. Sample size (n) refers to the number of individuals analysed after the 'NA' cases have been automatically removed during the analysis.

Population	FU9	FU13	FU15	FU17	FU22	Sample size (n)	**Correct (%)**
Moray Firth (FU9)	3	2	3	9	0	17	**52.94**
Firth of Clyde (FU13)	3	0	9	0	0	12	**0**
Irish Sea West (FU15)	3	3	31	4	0	41	**75.61**
Aran Grounds (FU17)	53	1	5	3	0	62	**85.48**
The Smalls (FU22)	2	0	0	2	0	4	**0**

4.6. Figures

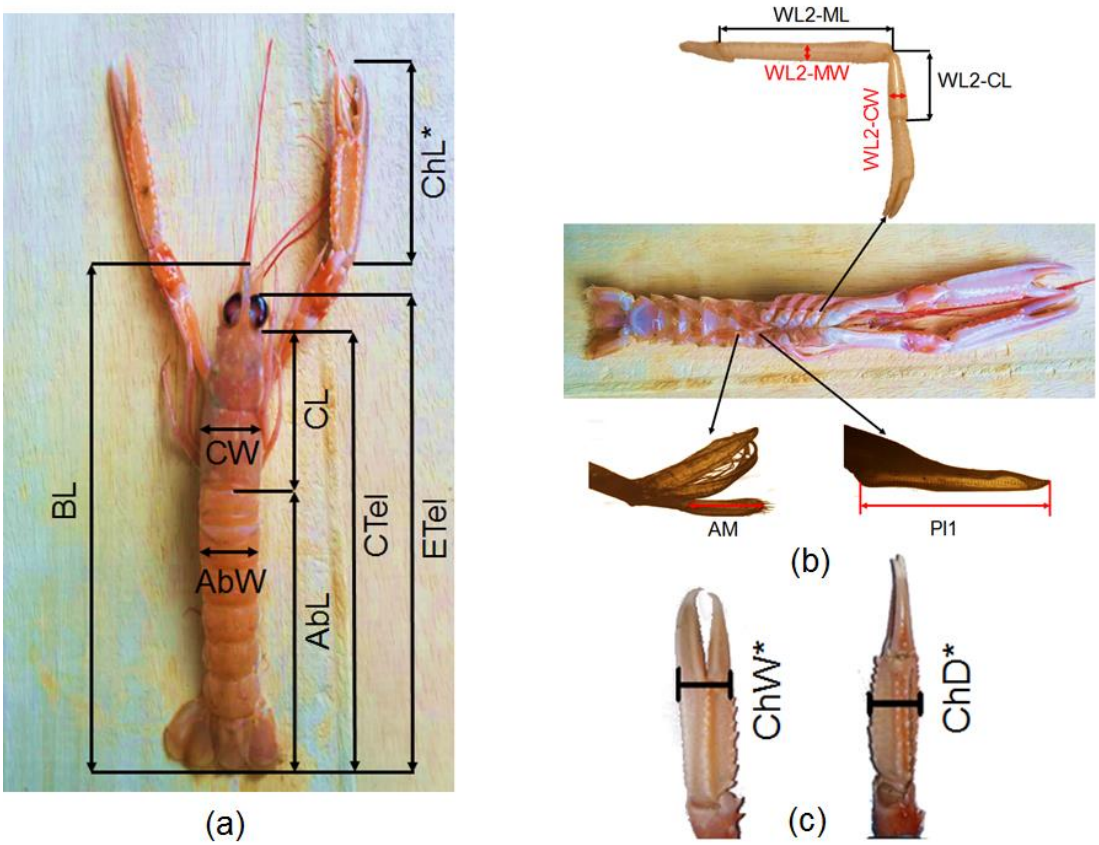

Figure 4.1. Body structure measurements considered in the study of variation in morphometric structure and morphometric size at the onset of maturity (=MSOM) of *Nephrops* populations across Irish and Scottish functional units: Moray Firth (FU9), Firth of Clyde (FU13), Western Irish Sea (FU15), Aran Grounds (FU17) and The Smalls (FU22). Full descriptions of body structures can be seen in table 4.1. Photo credits: Adrian Walsh and Conor Smyth.

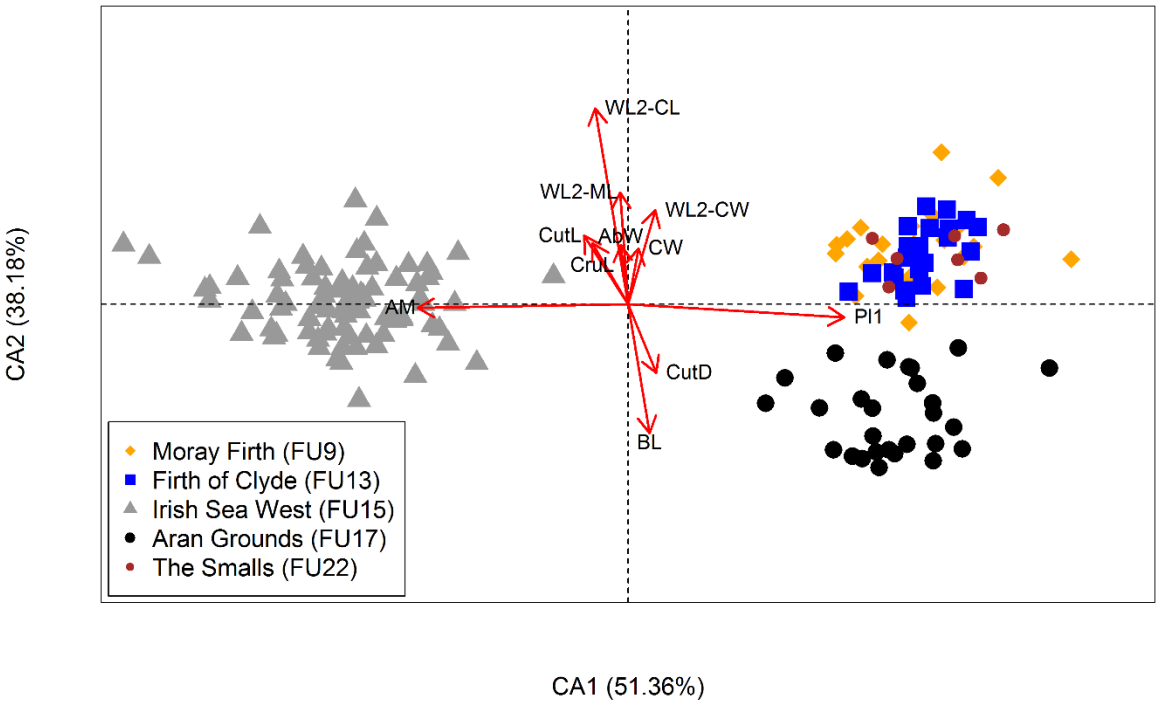

Figure 4.2. Plot of the morphometric scores obtained from the canonical discrimination analysis based on morphometric measurements of body structures in male *Nephrops* from: Moray Firth (FU9), Firth of Clyde (FU13), Western Irish Sea (FU15), Aran Grounds (FU17) and The Smalls (FU22). The proportion of variation explained by the different eigenvectors (CA1 and CA2 - 51.36 and 38.18% respectively) and significant body characters (BL, AbW, CW, CruL, CutL, CutD, WL2-ML, WL2-CL, WL2-CW, AM and Pl1) for morphometric discrimination are displayed in the plot.

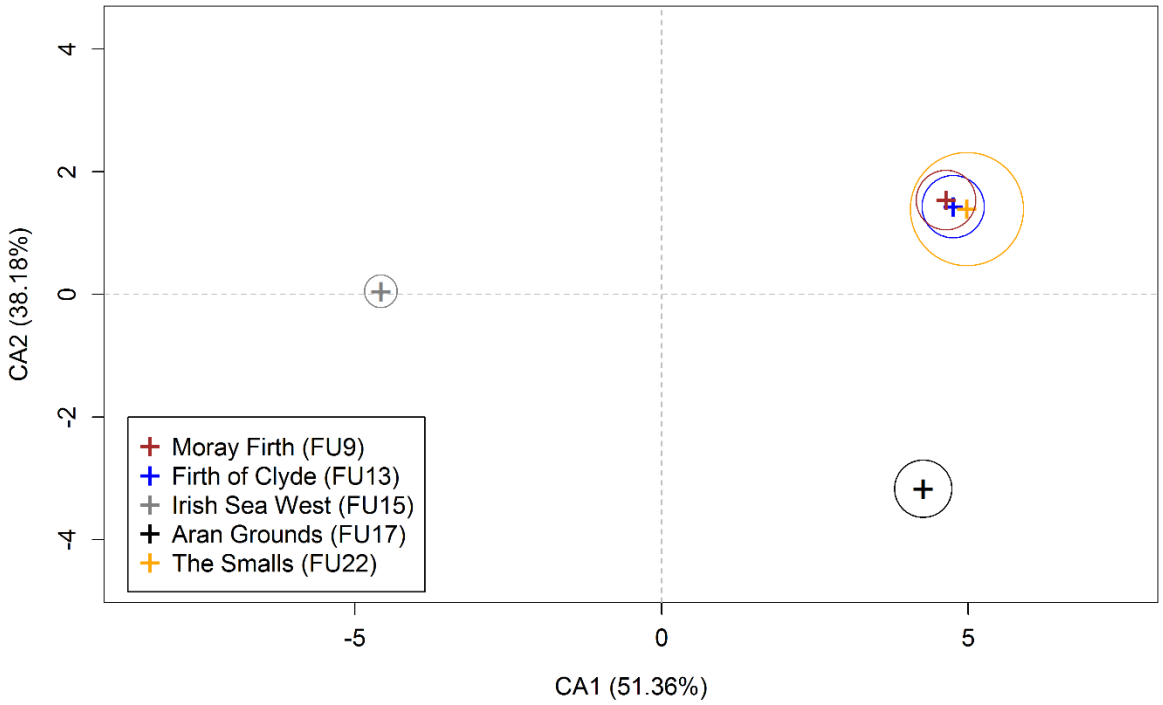

Figure 4.3. Plot of the centroids of morphometric scores with respective 95% confidence intervals obtained from the canonical discrimination analysis based on morphometric measurements of body structures in male *Nephrops* from: Moray Firth (FU9), Firth of Clyde (FU13), Western Irish Sea (FU15), Aran Grounds (FU17) and The Smalls (FU22). The proportion of variation explained by the significant eigenvectors (CA1 and CA2) are displayed in the plot (51.36 and 38.18 %, respectively).

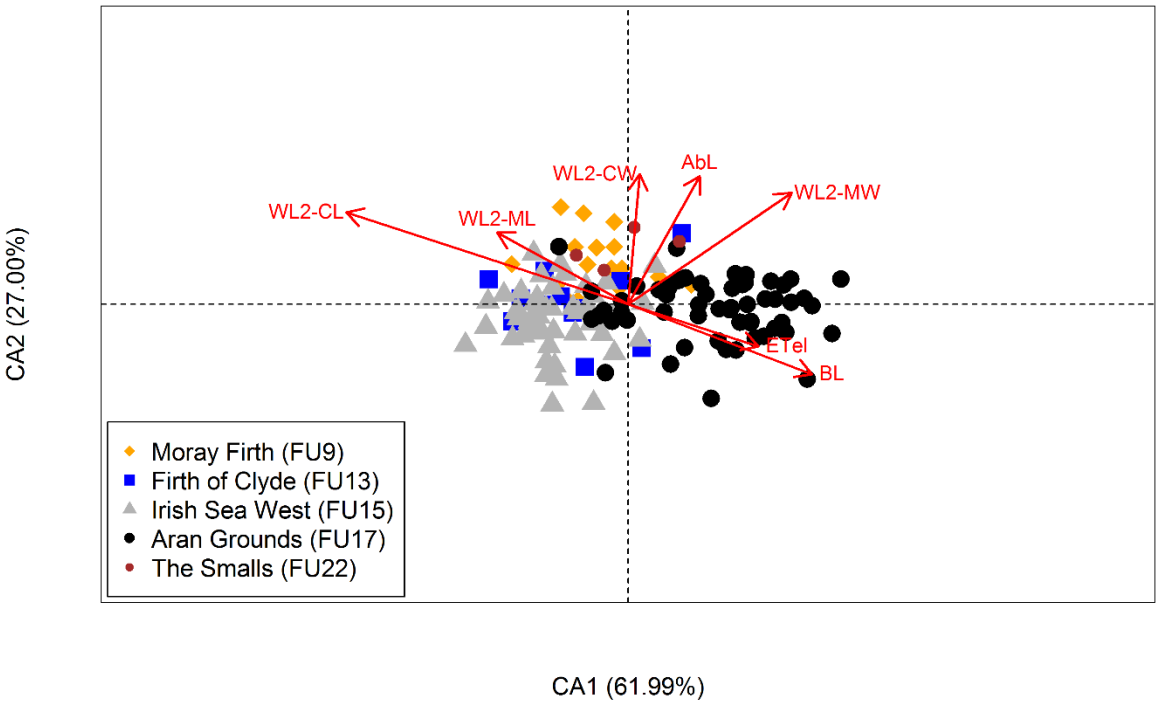

Figure 4.4. Plot of the morphometric scores obtained from the canonical discrimination analysis based on morphometric measurements of body structures in female *Nephrops* from a variety of Irish and Scottish functional units: Moray Firth (FU9), Firth of Clyde (FU13), Western Irish Sea (FU15), Aran Grounds (FU17) and The Smalls (FU22). The proportion of variation explained by the different eigenvectors (CA1 and CA2 - 61.99 and 27%, respectively) and significant body characters (BL, ETel, AbL, WL2-ML, WL2-MW, WL2-CL and WL2-CW) for morphometric discrimination are displayed in the plot.

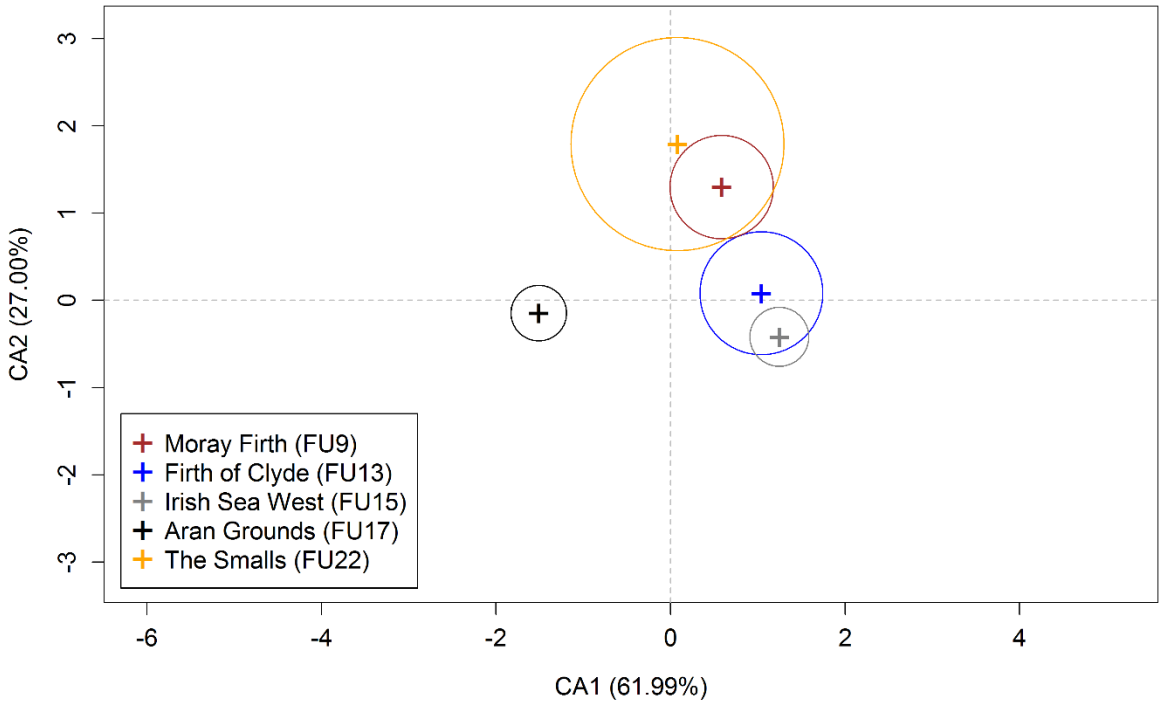

Figure 4.5. Plot of the centroids of morphometric scores obtained from the canonical discrimination analysis based on morphometric measurements of body structures in female *Nephrops* from: Moray Firth (FU9), Firth of Clyde (FU13), Western Irish Sea (FU15), Aran Grounds (FU17) and The Smalls (FU22). The proportion of variation explained by the significant eigenvectors (CA1 and CA2) are displayed in the plot (61.99 and 27%, respectively).

Chapter 5

General Discussion

5. General Discussion

5.1. Discussion

The research presented within this thesis contemplates two essential functions for the sustainability of biological populations: feeding and reproduction. The overall objective was to provide tools to examine diet and population maturity of *Nephrops norvegicus* on Irish fishing grounds.

In Chapter 2, stable isotope analysis within a Bayesian framework was chosen as a *'tool to assess Nephrops' diet'*. The choice was based on the fact that this approach is more appropriate to identify soft bodied prey, which are entirely destroyed by the digestive process, as well as microscopic suspended food such as plankton and suspended particulate organic matter, which would be difficult to identify by stomach content analysis. In addition, stable isotope analysis can show seasonal differences in the diet due to distinct turnover rates among different tissues such as muscle and hepatopancreas, that therefore represent different time snapshots in the diet of the individuals analysed. Key findings in this investigation about *Nephrops* feeding ecology include the importance of suspended particulate organic matter in the diet of male and female *Nephrops* of different sizes, which was suggested as a possible strategy for females to avoid starvation during the breeding period when they remain in burrows incubating their eggs (de Figueiredo and Thomas, 1967; Farmer, 1974c, Sardà, 1991). The research has also showed significant difference in the consumption of suspended particulate organic matter among individuals of different sizes, especially a higher consumption by smaller male individuals when compared to the larger ones. Thus, suspension feeding was also suggested as a possible strategy of smaller individuals to avoid antagonistic encounters (in the search for food) with aggressive and dominant larger males, especially in sites of high population density (Bell et al., 2013; Sbragaglia *et al.*, 2017; Merder *et al.*, 2019), as well as a feeding strategy of smaller individuals due to their inability in handling larger or more mobile prey. Sbragaglia *et al.*

(2017) has shown the existence of dominance hierarchies in male *Nephrops* in the laboratory-controlled environment, which showed increased burrow occupancy by larger-dominant individuals. This fact seems to contradict the hypothesis that a higher consumption of POM_{susp} among smaller individuals compared to larger ones is due to smaller *Nephrops* spending more time in burrows to avoid predation or antagonistic encounters with larger dominant individuals, however, Sbragaglia *et al.* (2017) emphasized that the laboratory environment in their experiment was extremely different from the one in the wild, which can be less competitive, considering that individuals can change burrows or even build new ones. In addition, Sbragaglia *et al.* (2017) stated that very little is known about *Nephrops* ecology in the field in relation to size and sex. However, the investigation described in Chapter 2 contributes to fill this gap, at least in relation to *Nephrops* feeding ecology in the wild. Overall, the high consumption of POM_{susp} observed in the stable isotopes analysis, irrespective of the size, sex, season or time interval considered in the investigation, provides evidence that *Nephrops* spend most of their time in burrows, either filter- or possibly even deposit feeding in an environment rich in organic matter, and that this species spends a much reduced period outside the burrow environment, to scavenge or forage mobile prey, moult, mate and fight for their burrows (Bell *et al.,* 2013).

In Chapter 3, the main aim was to develop a new *'tool to assess size at the onset of maturity'* in female *Nephrops*, which was called theoretical size at the onset of maturity (TSOM). Secondarily, this tool was applied to female *Nephrops* across a variety of Irish fishing grounds and the output compared to other maturity metrics, as well as linked with population density across these fishing grounds. The new methodology is very simple and less time consuming than other methodologies used for estimating size at maturity, since it uses data routinely collected in stock assessment exercises by national responsible organizations. It is important, because density-dependent size structure has been shown in crustacean species such as Norway

lobster, southern rock lobster, Oregon shore crab and sharp-nosed crab (Bailey and Chapman, 1983; Hines, 1989; Briggs, 1995; McGarvey *et al.*, 1999; Tuck *et al.*, 2000; Johnson *et al.*, 2013). Furthermore, for some crustacean species, this density-dependent size structure also seems to scale with SOM (Hines, 1989; Briggs, 1995; Tuck *et al.*, 2000; Queirós *et al.*, 2013), as well as with fishing pressure, e.g. in the rock lobster, although the genetic and ecological drivers in such cases are difficult to separate (Pollock, 1995). The new methodology is based on length-maturity data that allows it to be extrapolated to length-frequency data and even for the TSOM to be estimated when no maturity readings are available (see eq. 3.3: $TSOM = 3.15 + 0.63CL$), which is one of the key findings related to this new methodology, since it seems to address the fact that physiological-based methods tend to be prone to bias due to seasonality in the female reproductive cycle (Queirós *et al.*, 2013). Thus, TSOM can be calculated based on physiological maturity from 'baseline' surveys carried out at optimal times of year, and once baselines are established, in other years where this is not possible, e.g. where maturity surveys take place at imperfect times of year, or in years where surveys are disrupted or delayed for whatever reason, TSOM can be estimated from carapace length (CL). More broadly, the estimation of maturity from CL may also be very useful in meta-analyses focussed on onset of maturity, e.g. across crustaceans, or this could be used to generate hypotheses in macro-ecology.

Other key findings in Chapter 3 were the existence of well-defined linear relationships between the TSOM and L_{50} (which is the 'industrial standard' in routine use currently for estimation of size at maturity), and also with population density (see eq. 3.4: $L_{50} = 10.97 + 0.63TSOM$ and eq. 3.6: $TSOM = 24.35 - 4.93Density$). The latter allow both L_{50} and TSOM to be estimated *without* maturity readings and therefore can address the issue of a data gaps or potential seasonal biases related to the estimation of L_{50} (although, it must be stressed again that a baseline must first be established for these relationships to be used with any confidence).

Furthermore, the equations above-mentioned (eq. 3.4 and eq. 3.6) can provide L_{50} estimates when the traditional methodology (logistic regression model) fails, for various reasons, as outlined in Chapter 3.

In Chapter 4, the main objective was to examine some morphometric variables that may indicate onset of maturity ('MSOM'), based on different body structures in males and females, including one new structure, the male first pleopod that is modified for copulation. Some body structures, such as appendix masculina and male first pleopod (considered for the first time in this study), seemed to be good structures for MSOM estimation, since they provided significant estimates for all the FU / years studied, which were credible, and these were consistent with independent estimates for a couple of grounds in the North-eastern Atlantic. An example would be an MSOM of 25.09 mmCL (based on appendix masculina) and 25.63 mmCL (first pleopod) for Western Irish Sea which were similar to estimates reported by McQuaid *et al.* (2005, 2006) for this area (in the range 24.3-26.9 mmCL for appendix masculina across a variety of stations), as well as the estimate based on the appendix masculina for Firth of Clyde, described in Chapter 4, that was consistent with the estimate in Queirós *et al.* (2013) for the same body structure, both estimated at around 27 mmCL. Despite this, most of the MSOM results showed great variability within FU / year groups (CV > 5%, see table 3 and 4). The same issue was also noted in previous studies (Tuck *et al.*, 2000; McQuaid *et al.*, 2006; Queirós *et al.*, 2013). We believe that the variability in MSOM estimates, including variable allometric growth relationships (positive or negative) observed in the investigation, may be due to the inability of the method to identify the beginning and end of the period of allometric growth, as observed by Wynne (2016) in his work about Spotted Spiny Lobster (*Panulirus guttatus*) in Anguilla, British West Indies. Indeed, Conan *et al.* (2001) stated that growth stanzas in crustaceans may be too subtle that prevent MSOM to be identified by segmented regression models. For more reservations about segmented regression models and the influence of the dataset on the analysis

of allometric growth, see Clayton (1990). Secondary objectives in Chapter 4 were to examine possible morphometric discrimination of *Nephrops* populations at a variety of Irish and Scottish functional units, as well as to identify the main characters contributing to such discrimination, before finally investigating any link with population density across these grounds. The key finding of this section of the study demonstrated, for the first time, the existence of morphometric variability among *Nephrops* populations across sites in the North-eastern Atlantic. This was somewhat surprising, given the openness of these populations from a genetic viewpoint (Maltagliati *et al.*, 1998; Passamonti *et al.*, 1997; Stamatis *et al.*, 2004; Streiff *et al.*, 2001). Gallagher *et al.* (2018) have recently shown that there is no evidence of genetic structuring in Atlantic stocks. Indeed, because *Nephrops* are so distantly spread as larvae (very few are retained on the native grounds and wide dispersal is common, see McGeady *et al.*, 2020), there is no reason to assume that there are reproductive barriers in north Atlantic populations. Although the hypothesis about the existence of a relationship between the morphometric structure of *Nephrops* populations and population density across the grounds in the study was formally rejected, in fact, this relationship partially held, except for The Smalls (FU22) and Firth of Clyde (FU13). It is important to emphasize that the Firth of Clyde fishing grounds present a well-known density-size gradient operating within them, with low density across northern parts of these grounds and high density across southern ones (Tuck *et al.*, 1997a, b). In addition, other environmental conditions among the studied sites such as differences in alkalinity, current pattern, temperatures, turbidity, and environmental impacts could also affect morphometry (Marr, 1957; Maynou and Sardà, 1997; Siddik et al., 2016). Alternatively, these factors may co-vary with sediment type, the latter being tightly linked with *Nephrops* density because the proportion of mud (silt+clay) has a dome-shaped response with *Nephrops* density (Campbell *et al.*, 2009; Johnson *et al.*, 2013). Thus, a series of environmental

factors could appear to affect morphometrics *via* the ultimate factor: density, and its effects on morphometrics.

5.2. Future work

Recommendations for future research on the feeding ecology of *Nephrops norvegicus* (Chapter 2) include estimation of trophic enrichment factors (TEFs) for this species. Another research topic (thought during the research) would be the development of a package (possibly in R environment) that could suggest the best TEF based on the species considered in any particular diet investigation by stable isotope analysis, however, Healy *et al.* (2018) seemed to have the same insight and anticipated a R package that can suggest the best choice TEFs for mammals and birds, based on Bayesian inference that considers information on the tissue type and feeding ecology of the consumer. Perhaps this package might be extended for decapod crustaceans and other species. Additional topics of research could be to explain the reason for the differences between active and suspension feeding in long- and short-term storage tissues across the seasons considered in the study (Supplementary Fig. A2.1 in appendix 1), i.e., consumption of suspension feeding increased in short-term storage tissues, while higher active feeding was observed in long-term storage tissues (perhaps, this is due to inappropriate turnover rates considered in this study for these tissues what may be itself another topic for future work). The reason for a higher consumption of fish by smaller individuals when compared to the large ones in some occasions (Figure 2.2, Supplementary Table A2.3 in appendix 1) may also be a topic for further investigation. Furthermore, additional studies into seasonal comparisons are needed since this aspect of this work was relatively preliminary.

As stated before (see discussion in Chapter 2), future studies combining fatty acid analysis and SIA may further disentangle the various sources of organic particulates (e.g. Bosley et al., 2017) and their relative importance, including sources found on the benthos and inside lobster burrows. Furthermore, next generation sequencing (NGS) such as DNA metabarcoding may

be an important tool to complement SIA investigations and improve feeding ecology studies concerning *Nephrops* or other decapod species.

Finally, as discussed in Chapter 2, sediment organic matter (SOM) could be another important food source for benthic organisms like *Nephrops*, however there is a practical difficulty in distinguishing SOM from POM_{susp} because the latter eventually falls to the seafloor and therefore forms one component of the SOM. Therefore, there would be important future investigations to clarify the mechanism by which *Nephrops* feed on particulate material in the wild and answer questions such as: do *Nephrops* gather flocculant material, wait for the material to deposit in their burrows or actively filter feed? The answers for these questions might confirm the results obtained herein, that *Nephrops* can actively feed and that POM_{susp} is an important food source for this species, and further contribute to fully understanding the feeding ecology of the Dublin Bay prawn.

Future recommendations to develop the research described in Chapter 3 might be to improve the TSOM methodology developed for female *Nephrops*, for example, to verify the theoretical exponential distribution representing samples of immature individuals (for purpose of TSOM estimation) by comparing it with the actual probability distribution of immature individuals collected according to an adequate experimental design, one that is designed to obtain representative samples of immatures, i. e. from extremely small to larger immature individuals. The latter might be quite difficult; however, this would show the robustness of the theoretical exponential distribution of immatures to bring confidence for applications of the methodology in future research. Another potential research topic to verify / improve the robustness of the new methodology is more related to mathematics statistics, i.e. further investigation of the TSOM according to statistical theory including the topology of the TSOM interval with its specific properties. Additional topics might be to verify the validity of the methodology considering male *Nephrops*, other geographical areas with different abiotic parameters (e.g.

water temperature) and other taxonomic groups. Investigations might also be carried out to verify the utility of TSOM in calibrating more routinely used estimates e.g. L_{50}, as for example using Bayesian theory. Finally, another interesting topic concerning *Nephrops* maturity would be resorption of the ovary in females, since this process is not fully understood with gaps regarding how ovary resorption affects the fecundity and reproductive capacity of the stock, as well as what factors rule this process (see Becker et al., 2020).

Future recommendations to develop the research described in Chapter 4 might be investigations to clarify which factors, besides population density, play a role in determining the morphometric structure of *Nephrops* populations. Another topic would be to investigate the role of habitat usage in moulding the morphometric structure of *Nephrops* populations and, for this, the focus could be the entire biological community. For example, besides considering *Nephrops* population density as an isolated factor in the analysis, the inclusion of other factors such as the density of all organisms on the ground or population density of prey and predator should be considered. This last factor (predator density) together with the habitat characteristics can play an essential role moulding some morphological structures, since Gomes *et al.* (2016) have shown that the link between morphology and habitat use is mediated by refuge use. Perhaps the density of prey, together with habitat characteristics might be an important factor as well, because it can imply different strategies and usage of different body structures during the process of predation. To illustrate this point, consider a general ecological requirement for *Nephrops*: burrow building - perhaps the shape of appendages such as claws or walking legs might determine the difficulty of performing this ecological task. The shape of these structures, or other appendages, might also vary with the density of predators, or with the density of prey, or with sediment type. For example, will crusher morphology of *Nephrops* in a site rich in soft-bodied prey items be similar or different to that of *Nephrops* in a site rich in hard-shell prey? For research questions like this, I believe that an ecological approach considering interaction

among individuals of the entire biological community (including abiotic variables) will be important for obtaining robust results.

As discussed in Chapter 4, there are some issues concerning the estimation of the MSOM, such as a great variability in the results and inconsistencies between allometry and behaviour of the relative growth of body structures to CL after MSOM, which are potentially linked to a weakness in the methods currently used to determine the type of allometric growth of body structures, as well as to estimate the morphometrical size at the onset of maturity. Therefore, it would be very important for future research to investigate the best type of statistical model for estimating reliably the type of allometry and MSOM in *Nephrops*. Furthermore, heterogeneity in the morphometric structure of *Nephrops* populations across the North-eastern Atlantic, evidenced in the preliminary study reported in Chapter 4, suggests the need for further studies on the drivers of morphometric variation in *Nephrops*. It would be important that these investigations will include abiotic variables such as temperature, salinity, density, type of sediment, as well as other methods of classification (e.g. CAP, Random Forest or CART), which might allow to establish a phenotype-environment correlation for the morphometric structures, as well as to improve the discrimination and classification success concerning the morphometric structure of *Nephrops* at the grounds in the study.

Finally, we believe that, for *Nephrops*, population density is an over-arching theme, and everything needs to be evaluated in this context. McGeady (2020) has shown that recruitment variation on various grounds due to supply-side issues (including larval retention and larval imports from outside) can be linked to adult density at low-recruitment grounds. Added to this is how the recruiting population responds to the sediment type (i.e. once the larvae have arrived, how many *Nephrops* each ground can support). It would be interesting to compare sediment granulometry (including silt+clay content) across all the European FUs in *Nephrops*, to see

how well this correlates with density as part of a larger study. It is already known that density scales with body size across the European FUs (Briggs, 1995; Tuck *et al.*, 2000; Johnson *et al.*, 2013) but how this scales with silt+clay content would add further understanding to the ecological requirements of *Nephrops* across the range.

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7. Appendices

7.1. Appendix 1 – Supplementary Material for Chapter 2

Table A2.1. Putative macrofaunal prey species and tissue type analysed for stable-isotope ratios, along with mean isotopic signatures.

Group	Species	Nº samples	Tissue type analysed	Group mean ± SD $\delta^{13}C$	Group mean ± SD $\delta^{15}N$
Sampling date 1: 29-May-2014					
Filter feeders	*Turritella communis*	3	Foot muscle	-17.2 ± 1.29	9.9 ± 0.70
	Aequipecten opercularis	4	Adductor muscle		
	Tunicate sp.	2	Whole body		
	Terebellidae sp.	1	Whole body		
	Pecten maximus	4	Adductor muscle		
	Ostrea edulis	4	Adductor muscle		
Polychaete	*Nephtydiae sp.*	4	Whole body	-15.90 ± 0.64	11.24 ± 0.03
Crustaceans	*Liocarcinus depurator*	4	Cheliped muscle	-15.47 ± 0.80	13.00 ± 0.44
	Pagurus bernhardus	4	Cheliped muscle		
	Necora puber	4	Cheliped muscle		
	Carcinus maenas	4	Cheliped muscle		
	Palaemon serratus	4	Cheliped muscle		
	Crangon crangon	4	Cheliped muscle		
	Cancer pagurus	1	Cheliped muscle		
Fish	*Trisopterus minutus*	1	Dorsal muscle	-16.57 ± 0.34	13.75 ± 0.70
	H. platessoides	1	Dorsal muscle		
	Limanda limanda	4	Dorsal muscle		
	Callionymus lyra	4	Dorsal muscle		
	Merlangius merlangus	4	Dorsal muscle		
Sampling date 2: 25-July-2014					
Filter feeders	*Turritella communis*	3	Foot muscle	-17.85 ± 0.84	9.35 ± 0.47
	Aequipecten opercularis	4	Adductor muscle		
	Tunicate sp.	2	Whole body		
	Terebellidae sp.	3	Whole body		
	Anomia ephippium	4	Adductor muscle		
Polychaete	*Nephtydiae sp.*	4	Whole body	-14.58 ± 0.22	11.28 ± 0.72
Crustaceans	*Liocarcinus depurator*	4	Cheliped muscle	-15.49 ± 0.59	13.05 ± 0.48
	Pagurus bernhardus	4	Cheliped muscle		
	Necora puber	4	Cheliped muscle		
	Carcinus maenas	4	Cheliped muscle		
	Palaemon serratus	4	Cheliped muscle		
	Crangon crangon	4	Cheliped muscle		
	Cancer pagurus	4	Cheliped muscle		
Fish	*Trisopterus minutus*	4	Dorsal muscle	-17.22 ± 0.41	13.03 ± 0.56
	H. platessoides	4	Dorsal muscle		
	Merlangius merlangus	4	Dorsal muscle		
	Callionymus lyra	4	Dorsal muscle		

Table A2.2. *Nephrops* size classes of males and females sampled on 29th May and 25th July 2014 for stable isotope analysis. Size classes were defined based on the range of carapace lengths of the samples (27.30-58.10 mm). Small individuals were defined as the ones with CL ≤ 36 mm, large individuals were the ones with CL ≥ 44 mm and medium were the ones with CL between these values (36-44 mm).

Sampling date	Sex	Size class	Range of Carapace length (mm)
29-May-2014	Male	Small	27.30-34.00
		Medium	37.30-43.70
		Large	45.20-58.10
	Female	Small	30.70-35.30
		Medium	39.00-43.30
		Large	44.60-50.40
25-July-2014	Male	Small	27.50-35.80
		Medium	38.40-41.40
		Large	48.20-56.50
	Female	Small	27.70-33.70
		Medium	36.40-42.70
		Large	44.00-55.10

Table A2.3. Average contributions of the main food sources to *Nephrops* diet at Clew Bay, in different periods: *'Spring long'* (8th March-29th May 2014), *'Spring short'* (10th -29th May 2014), *'Summer long'* (4th May-25th July 2014) and *'Summer short'* (6th-25th July 2014). POM = suspended Particulate Organic Matter.

Spring long	Male			Female		
	Small	Medium	Large	Small	Medium	Large
POM	35.3%	26.1%	16.8%	24.5%	22.9%	19.4%
Phytoplankton	2.3%	4.8%	4.3%	5.5%	4.2%	5.3%
Zooplankton	2.8%	5.2%	5.4%	5.5%	5.3%	6.5%
Suspension feeding	**40.4%**	**36.1%**	**26.5%**	**35.5%**	**32.4%**	**31.2%**
Filter feeders	3.1%	5.9%	6.4%	6.2%	6.1%	7.5%
Polychaetes	3.4%	7.4%	7.5%	12.2%	7.1%	8.7%
Crustaceans	5.0%	10.4%	15.6%	15.2%	12.3%	18.4%
Fish	48.1%	40.2%	44.0%	30.9%	42.1%	34.2%
Active feeding	**59.6%**	**63.9%**	**73.5%**	**64.5%**	**67.6%**	**68.8%**

Spring short	Male			Female		
	Small	Medium	Large	Small	Medium	Large
POM	47.4%	35.7%	32.0%	47.4%	40.5%	33.5%
Phytoplankton	4.6%	5.6%	5.9%	3.6%	5.2%	5.4%
Zooplankton	5.5%	6.8%	7.4%	4.4%	6.3%	6.4%
Suspension feeding	**57.5%**	**48.1%**	**45.3%**	**55.4%**	**52.0%**	**45.3%**
Filter feeders	6.6%	8.1%	8.6%	5.2%	7.6%	7.5%
Polychaetes	7.1%	8.7%	9.0%	5.7%	8.3%	8.4%
Crustaceans	10.6%	14.1%	14.6%	10.6%	13.1%	13.6%
Fish	18.2%	21.0%	22.5%	23.1%	19.0%	25.2%
Active feeding	**42.5%**	**51.9%**	**54.7%**	**44.6%**	**48.0%**	**54.7%**

Summer long	Male			Female		
	Small	Medium	Large	Small	Medium	Large
POM	21.9%	19.1%	12.0%	22.7%	20.0%	20.6%
Phytoplankton	5.3%	5.5%	5.9%	5.5%	5.2%	3.7%
Zooplankton	5.4%	5.9%	5.9%	5.9%	5.7%	3.8%
Suspension feeding	**32.6%**	**30.5%**	**23.8%**	**34.1%**	**30.9%**	**28.1%**
Filter feeders	3.9%	4.0%	4.2%	3.9%	3.8%	2.7%
Polychaetes	3.9%	4.1%	4.2%	3.7%	3.5%	2.7%
Crustaceans	7.6%	8.6%	7.0%	9.9%	8.5%	5.6%
Fish	52.0%	52.8%	60.8%	48.4%	53.3%	60.9%
Active feeding	**67.4%**	**69.5%**	**76.2%**	**65.9%**	**69.1%**	**71.9%**

Summer short	Male			Female		
	Small	Medium	Large	Small	Medium	Large
POM	37.7%	39.6%	28.5%	40.7%	37.0%	34.1%
Phytoplankton	3.6%	4.4%	9.5%	3.9%	3.7%	4.3%
Zooplankton	3.7%	4.5%	9.8%	4.1%	4.0%	4.5%
Suspension feeding	**45.0%**	**48.5%**	**47.8%**	**48.7%**	**44.7%**	**42.9%**
Filter feeders	2.7%	3.3%	6.2%	3.0%	2.9%	3.3%
Polychaetes	2.8%	3.3%	5.5%	3.2%	3.0%	3.4%
Crustaceans	4.8%	6.1%	12.4%	5.4%	5.8%	6.8%
Fish	44.7%	38.8%	28.1%	39.7%	43.6%	43.6%
Active feeding	**55.0%**	**51.5%**	**52.2%**	**51.3%**	**55.3%**	**57.1%**

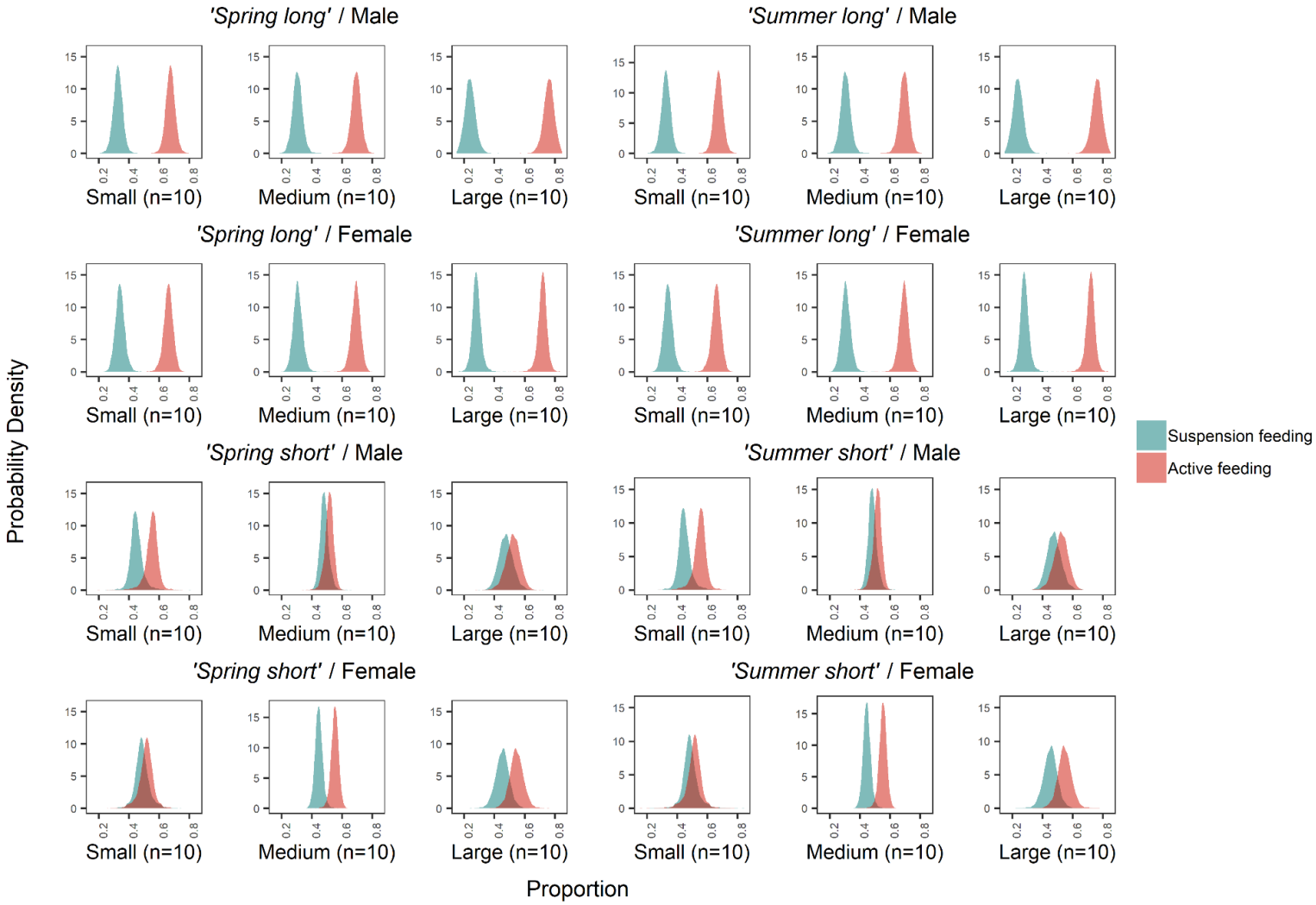

Figure A2.1. Probability distributions of the contributions of active and suspension feeding to the diet of *Nephrops* in different periods during Spring and Summer 2014: *'Spring long'* (8th March - 29th May 2014), *'Spring short'* (10th - 29th May 2014), *'Summer long'* (4th May - 25th July 2014) and *'Summer short'* (6th - 25th July 2014)

Supplementary Methods A2.1

Further details about stable isotope analysis

Isotope analysis was carried out at carried out at the Stable Isotope Core Laboratory of Washington State University (see also main text). The samples were converted into N_2 and CO_2 and separated with a 3 m gas chromatography (GC) column connected to a continuous flow isotope ratio mass spectrometer (Delta PlusXP, Thermofinnigan, Bremen) and Stable Isotope Ratios (R) were expressed in δ notations as parts per thousand (‰) using the following equation:

$$\delta X = \left(R_{\text{sample}}/R_{\text{standard}} - 1\right) \quad \text{Eq. (A2.1)}$$

The internationally accepted standards for carbon and nitrogen were: Vienna Pee Dee Belemite and atmospheric nitrogen, respectively. Samples were normalised through internal running standards (acetanilide and keratin), which were previously calibrated using sucrose reference material and was shown to be precise (mean ± SD: - 26.92 ± 0.07 for $\delta^{13}C$ and 6.04 ± 0.12 for $\delta^{15}N$).

Further details about residence time (*rt*) estimates

Sometimes isotopic residence times (= '*rt*') for different tissues can be obtained directly from the literature. For example, the *rt* of the ^{13}C and ^{15}N isotope signatures in muscle tissue of *Nephrops* was estimated from mantis shrimp[2] (also a decapod) and defined to be 81.05 days, which was the mean of the *rt* values for ^{13}C and ^{15}N residence times in that study. When literature provides only estimates of values for isotopic half-lives ($t_{1/2}$) in the tissue or tissue-specific turnover rates (λ), rt can be estimated, by the following equations (Thomas and Crowther, 2015):

$$rt = t_{1/2}/ln2 \quad \text{Eq. (A2.2)}$$

$$rt = 1/\lambda \quad \text{Eq. (A2.3)}$$

As the mantis shrimp study (deVries *et al.*, 2015) used above only considered muscle and haemolymph tissues, the value for the residence time of *Nephrops* hepatopancreas tissue was estimated from the ^{13}C half-life of *Callinectes sapidus* (blue crab) (Vedral, 2012) i.e. a half-life of 13.4 days. From this value, a residence time of 19.3 days could be derived from Eq. A2.2 above and, as they are both decapods, this value was also used for residence time of

Nephrops hepatopancreas tissue. Finally, as there were two tissue types and two sampling days, four time-related sampling intervals were defined (see Table 2.1 of main text).

Code and other material

The code and other files concerning Chapter 2 and output not included herein will be available at https://github.com/Caesar-Santana/PhD_Thesis_Scripts.git.

Supplementary References for Chapter 2

deVries, M. S., del Rio, C. M., Tunstall, T. S., Dawson, T. E. Isotopic incorporation rates and discrimination factors in mantis shrimp crustaceans. *PLoS One* **10(4),** 1-16 (2015).

Thomas, S. M., Crowther, T. W. Predicting rates of isotopic turnover across the animal kingdom: a synthesis of existing data. *J. Anim. Ecol.* **84,** 861-870 (2015).

Vedral, A. J. Blue crab residency and migration in the Mobile Bay estuary: a stable isotope study investigating connectivity (PhD Thesis). University of Alabama (2012). Available at: http://acumen.lib.ua.edu/content/u0015/0000001/0001038/u0015_0000001_0001038.pdf (Accessed: 01/09/2019).

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7.2. Appendix 2 – Supplementary Material for Chapter 3

Supplementary Methods A3.1

Simulation of TSOM relationship with average carapace length and L_{50}

Logistic models were used to simulate different samples of mature female *Nephrops* from normal distributions built with randomly generated values for the mean and standard deviation of the hypothetical samples of *Nephrops* females. The simulations were carried out as follows: 100 different values for L_1 and L_{50} (respectively, the size class at which 1 and 50% of the females are sexually mature) were generated randomly, as well as 100 values for the mean and standard deviation, each pair of values corresponding to a different hypothetical sample of *Nephrops* females (immature and mature). Based on the randomly generated values for L_1 and L_{50}, the parameters α and β of different logistic models (eq. A3.1) could be calculated:

$$\ln\left(\frac{p}{1-p}\right) = \alpha + \beta X \text{ (eq. A3.1)}$$

Where p, α, β and X are respectively the proportion mature in any size class, parameters of the logistic regression (α and β) and size class of the sample. These models were used to simulate 100 different samples of mature female *Nephrops* from the hypothetical samples of *Nephrops* females above-mentioned. After that, TSOMs were estimated according to the methodology described in previous sections of this study. Finally, a linear model was fitted between the TSOM and L_{50} metrics.

Code and other material

The code and other files concerning Chapter 3 and output not included herein will be available at https://github.com/Caesar-Santana/PhD_Thesis_Scripts.git.

Table A3.1. Annual population density (burrows m^{-2}) obtained from annual underwater television (UWTV) surveys carried out across Irish functional units: Western Irish Sea (FU15), Porcupine Bank (FU16), Aran Grounds (FU17), South Coast (FU19), Labadie, Jones and Cockburn Banks (FU2021) and The Smalls (FU22).

Year	FU15	FU16	FU17	FU19	FU2021	FU22
2001	-	-	-	-	-	-
2002	-	-	0.79	-	-	-
2003	0.99	-	0.94	-	-	-
2004	1.00	-	1.08	-	-	-
2005	1.02	-	0.81	-	-	-
2006	0.97	-	0.46	0.21	0.44	0.49
2007	0.93	-	0.69	-	-	0.37
2008	0.77	-	0.41	-	-	0.36
2009	0.83	-	0.52	-	-	0.36
2010	0.90	-	0.63	-	-	0.37
2011	0.88	-	0.51	0.34	-	0.41
2012	0.91	0.16	0.33	0.30	0.57	0.49
2013	0.78	0.11	0.33	0.25	0.16	0.41
2014	0.83	0.10	0.28	0.32	0.19	0.53
2015	0.79	-	0.40	0.24	0.20	0.49
2016	0.84	0.12	0.29	0.20	0.18	0.31
2017	0.90	0.09	0.31	0.25	0.44	0.55
2018	0.85	0.13	0.40	0.09	0.27	0.31

Table A3.2. Summary of linear model fit revealing a significant positive relationship between annual TSOM estimates and average carapace length of female *Nephrops*' (immature and mature) across all functional management units (FUs) considered in the study.

Variable	Coefficient	Std. Error	t-value	p-value
Intercept	3.15	1.02	3.08	0.003
Density	0.63	0.03	18.66	< 0.001
R-square	0.84	Adjusted R-squared	0.84	
F-statistic	348.1	Degrees of freedom	67	
p-value	< 0.001	Residuals std. error	0.99	

Table A3.3. Summary of linear model fit revealing a significant positive relationship between annual L_{50} and TSOM estimates of female *Nephrops*' across all functional management units (FUs) considered in the study.

Variable	Coefficient	Std. Error	t-value	p-value
Intercept	10.97	2.09	5.26	< 0.001
Density	0.63	0.09	6.71	< 0.001
R-square	0.40	Adjusted R-squared	0.40	
F-statistic	45.02	Degrees of freedom	67	
p-value	< 0.001	Residuals std. error	1.89	

Table A3.4. Summary of linear model fit confirming a significant relationship between L_{50} and TSOM by simulations carried out in the study.

Variable	Coefficient	Std. Error	t-value	p-value
Intercept	-1.37	1.13	-1.21	0.23
Density	1.16	0.05	25.27	< 0.001
R-square	0.87	Adjusted R-squared	0.87	
F-statistic	638.4	Degrees of freedom	95	
p-value	< 0.001	Residuals std. error	1.64	

Table A3.5. Summary of linear model fit revealing a significant inverse relationship between annual estimates of female *Nephrops*' TSOM and population density across all functional management units (FUs) considered in the study.

Variable	Coefficient	Std. Error	t-value	p-value
Intercept	24.35	0.31	79.55	< 0.001
Density	-4.93	0.46	-10.65	< 0.001
R-square	0.60	Adjusted R-squared	0.60	
F-statistic	113.4	Degrees of freedom	76	
p-value	< 0.001	Residuals std. error	1.24	

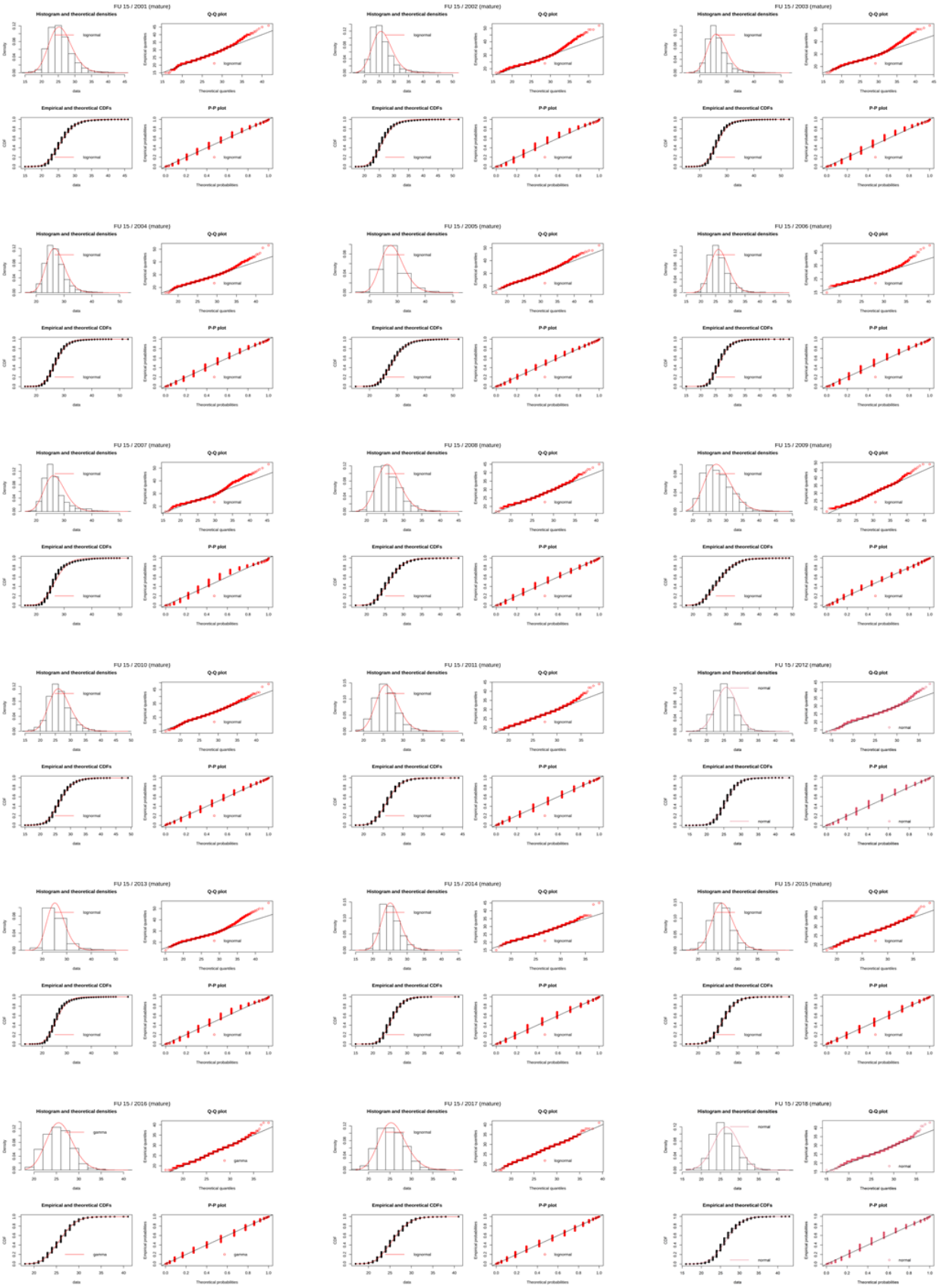

Figure A3.1. Fitted probability distributions of carapace lengths of mature females in Western Irish Sea (FU15) throughout 2001-18 and respective goodness-of-fit plots.

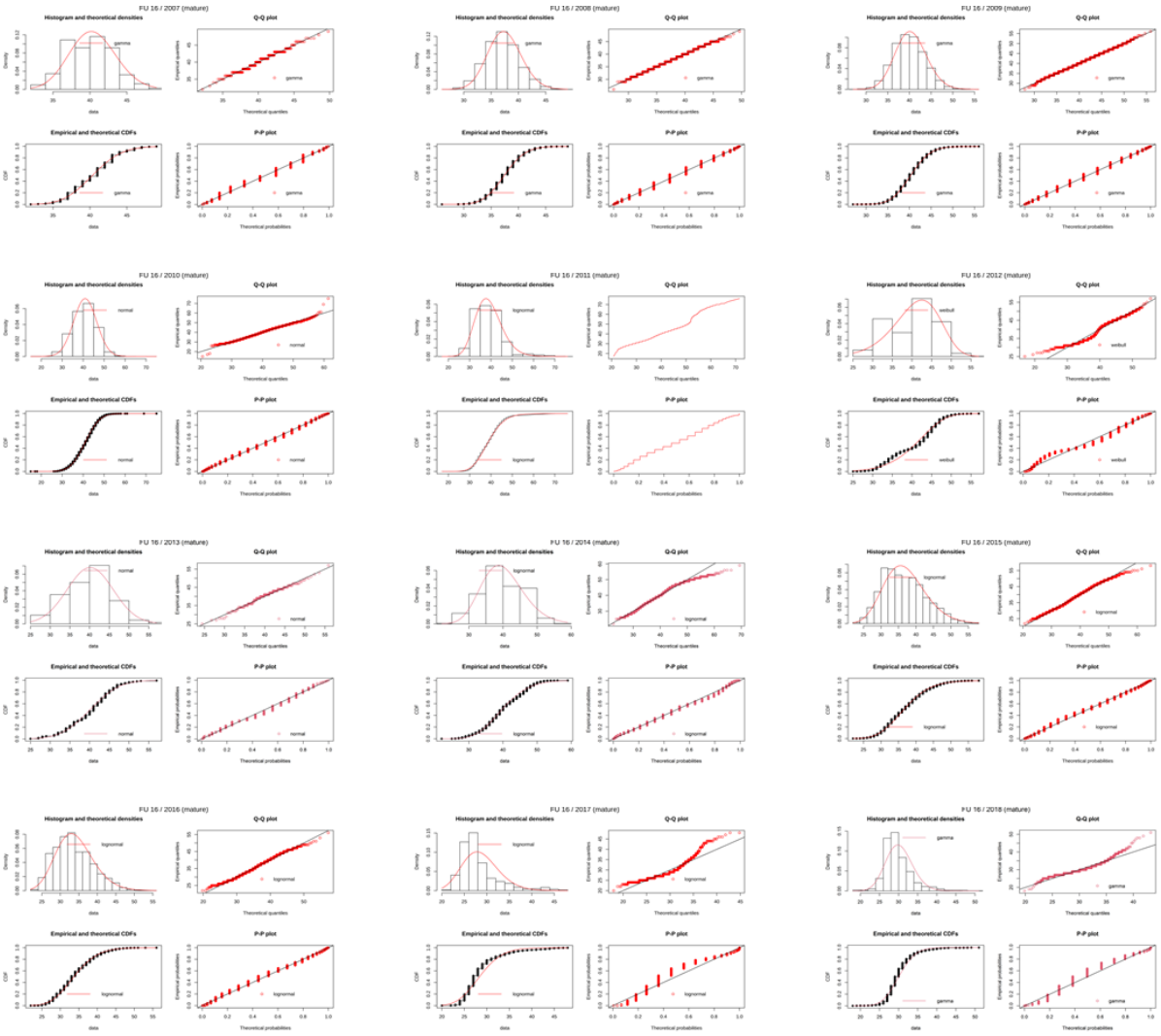

Figure A3.2. Fitted probability distributions of carapace lengths of mature females in Porcupine Bank (FU16) throughout 2007-18 and respective goodness-of-fit plots.

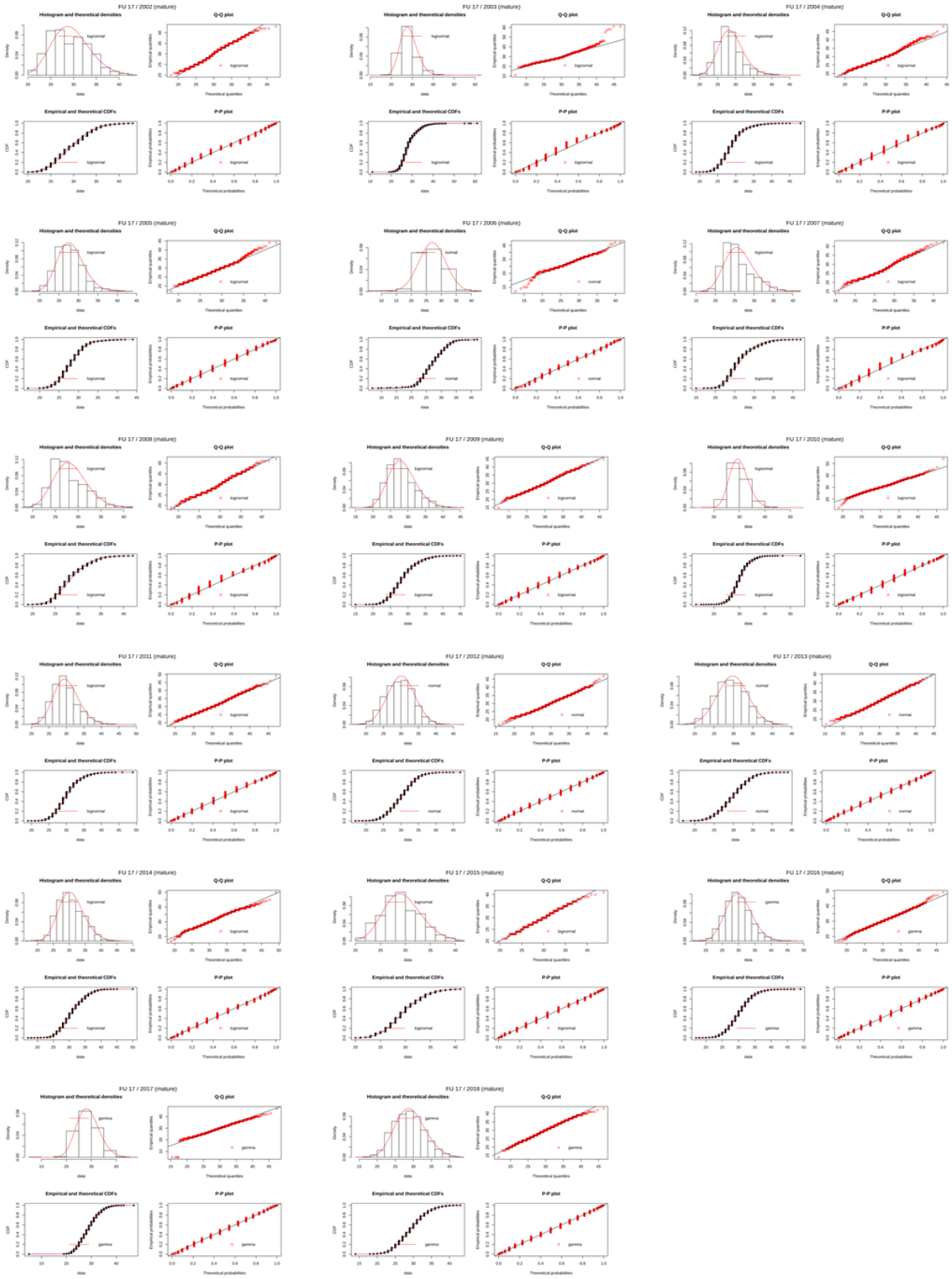

Figure A3.3. Fitted probability distributions of carapace lengths of mature females in Aran Grounds (FU17) throughout 2002-18 and respective goodness-of-fit plots.

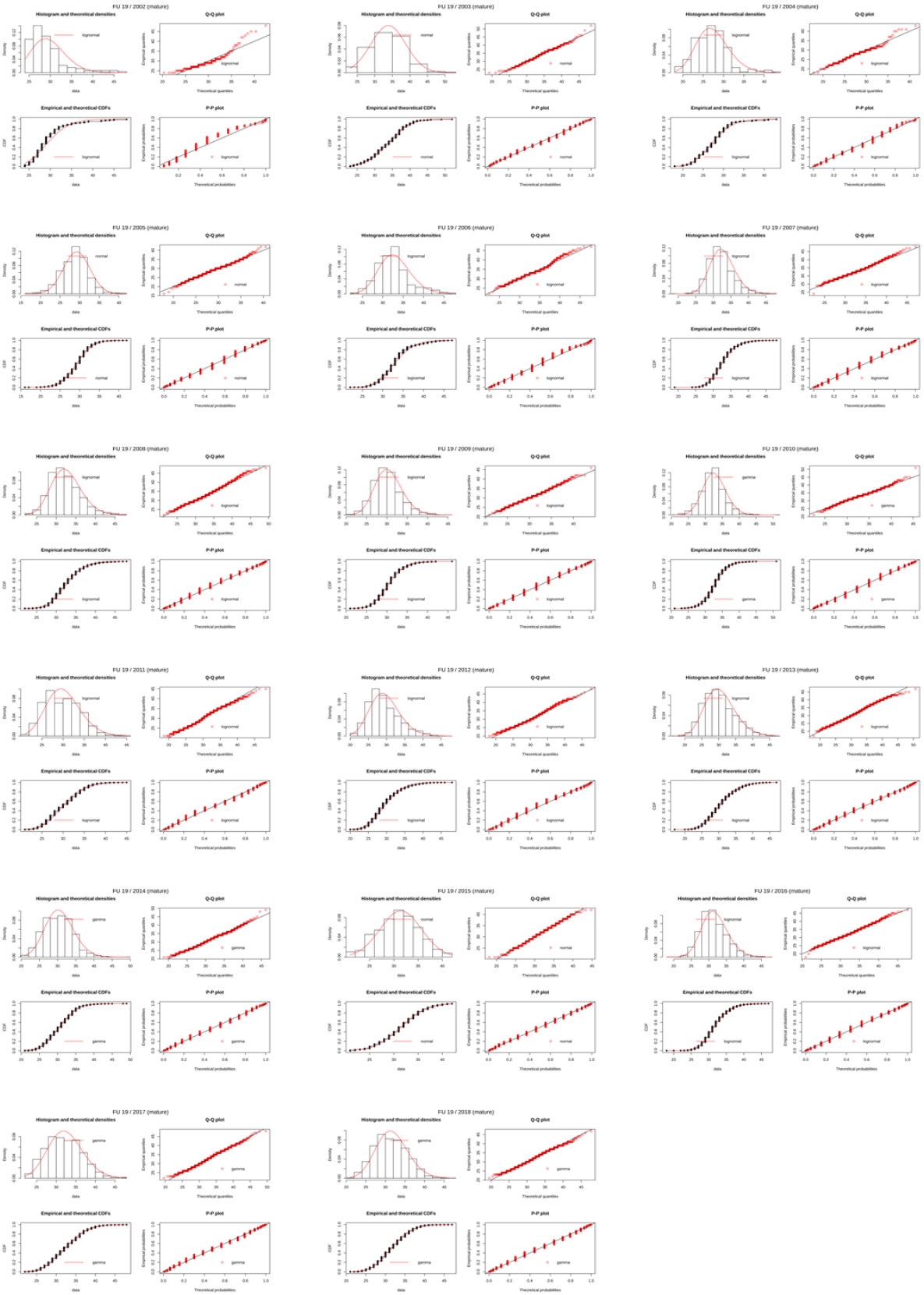

Figure A3.4. Fitted probability distributions of carapace lengths of mature females in South Coast (FU19) throughout 2002-18 and respective goodness-of-fit plots.

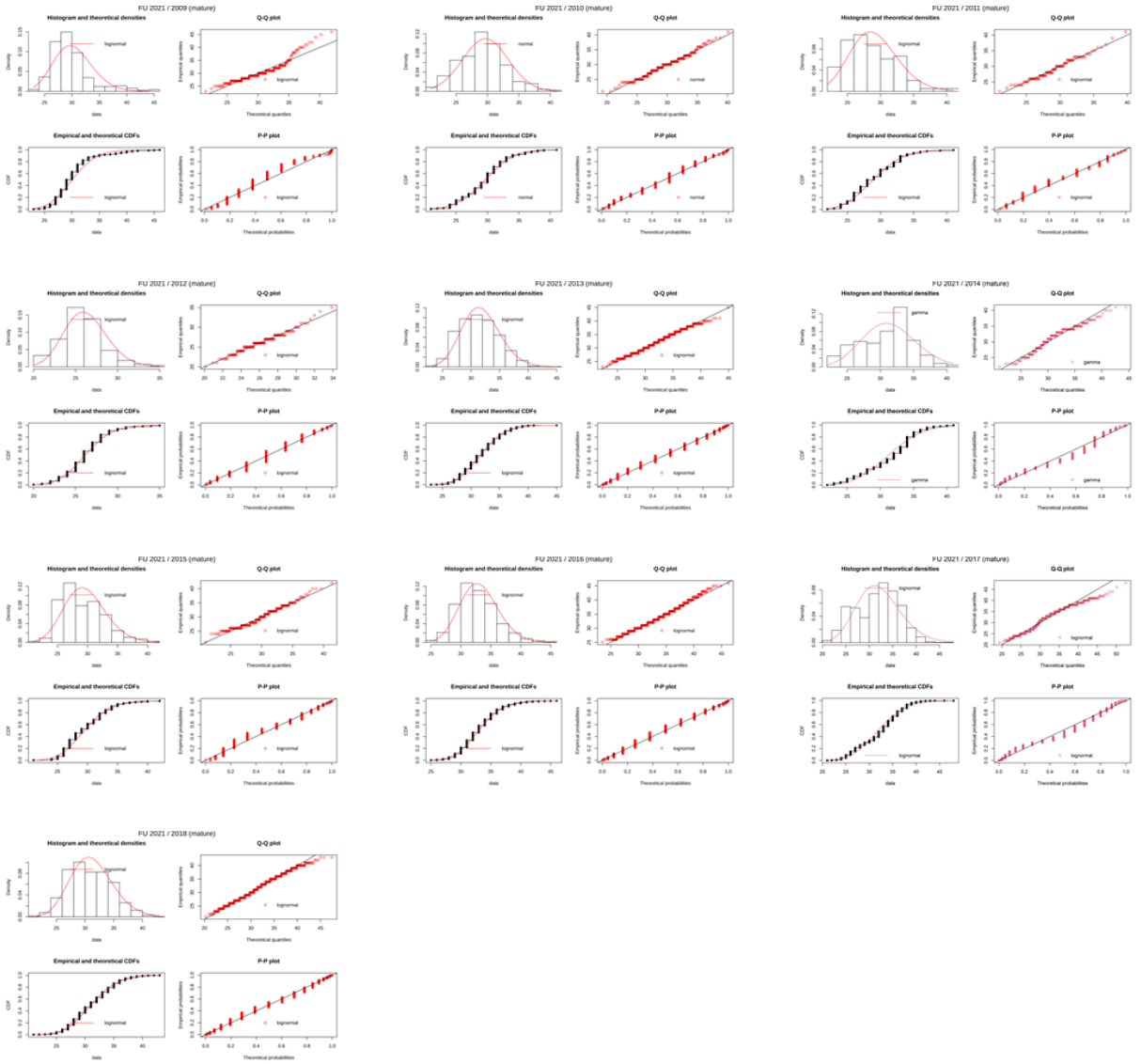

Figure A3.5. Fitted probability distributions of carapace lengths of mature females in Labadie, Jones and Cockburn Banks (FU2021) throughout 2009-18 and respective goodness-of-fit plots

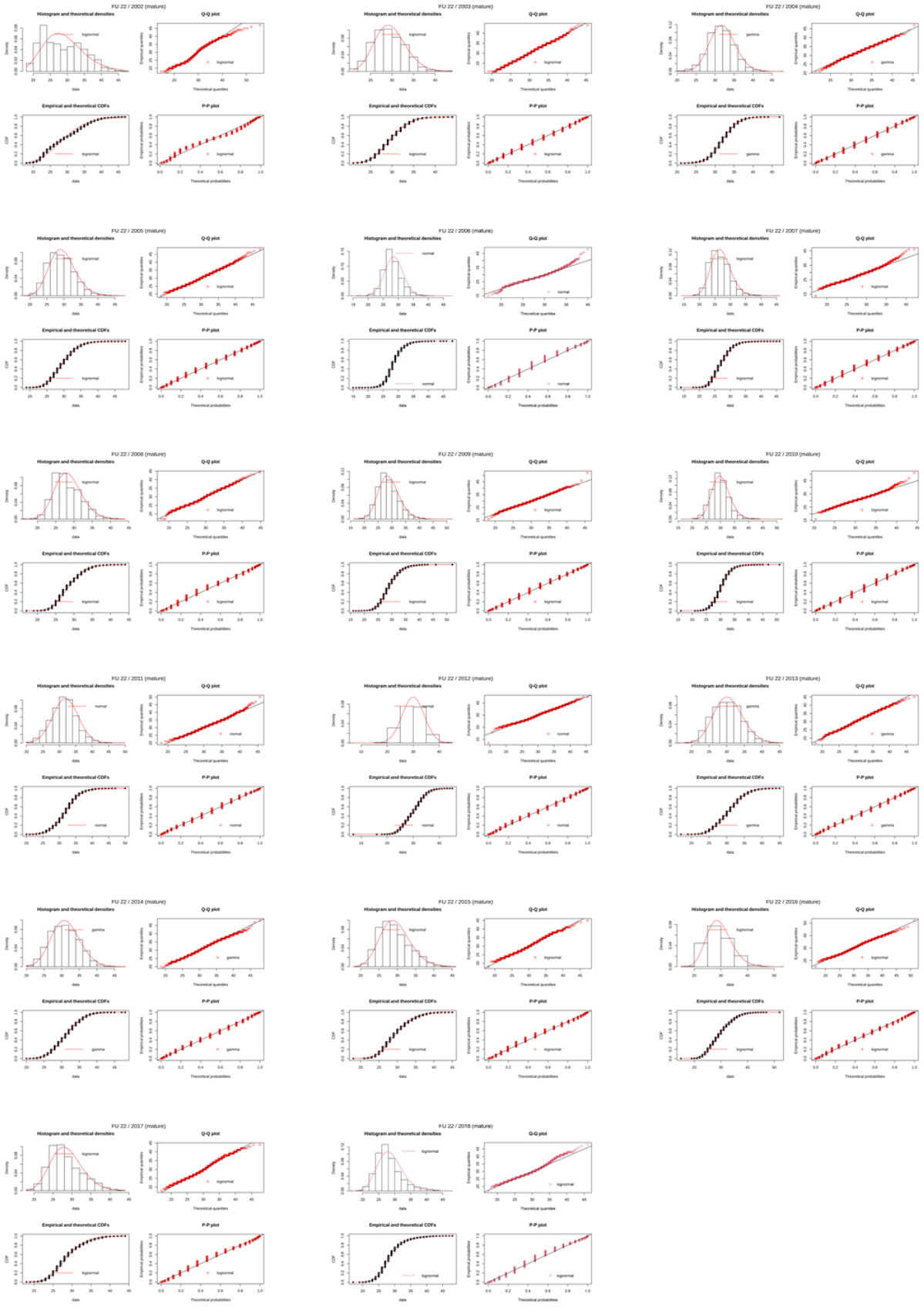

Figure A3.6. Fitted probability distributions of carapace lengths of mature females in The Smalls (FU22) throughout 2002-18 and respective goodness-of-fit plot.

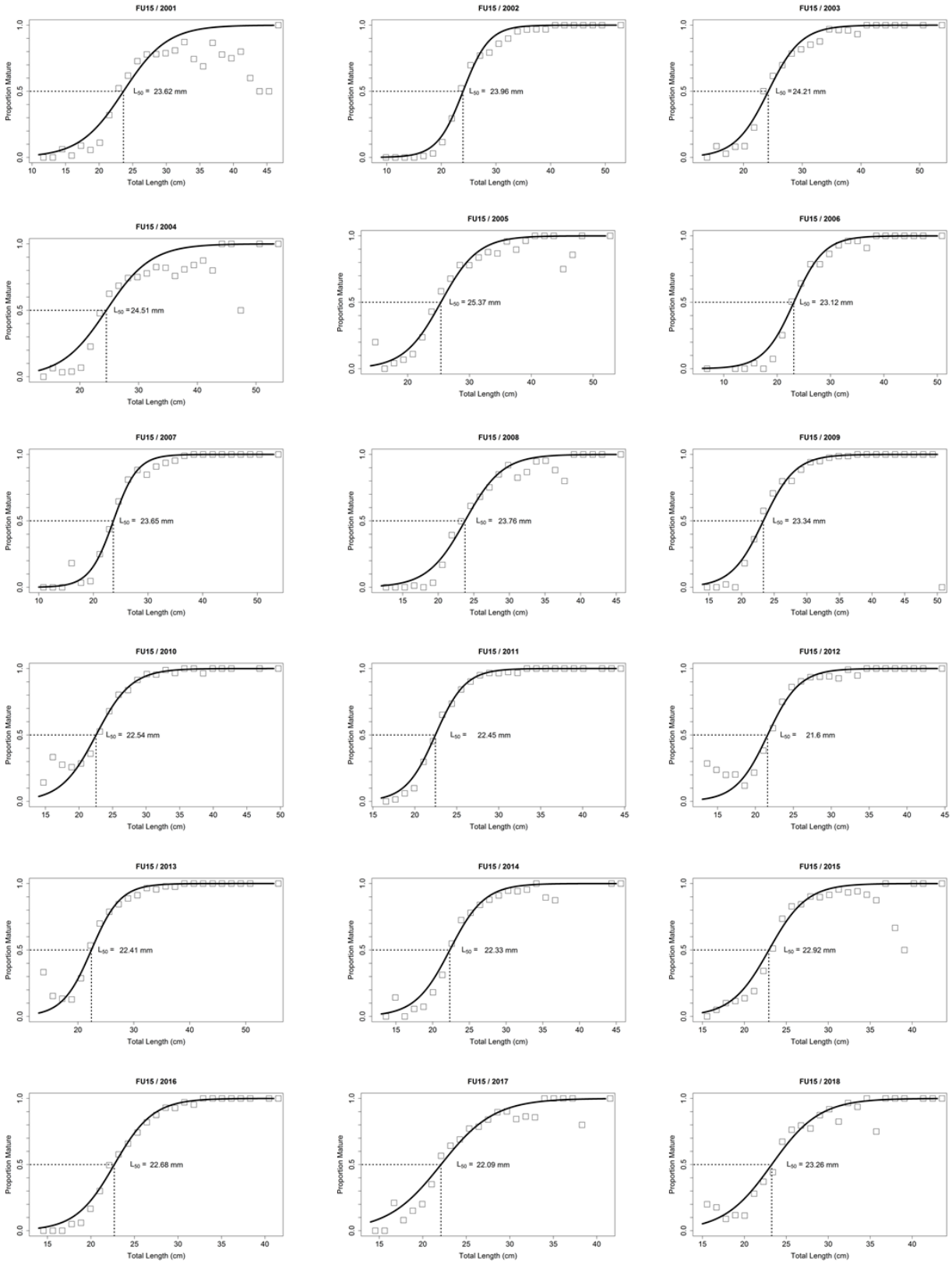

Figure A3.7. L_{50} estimates from maturity ogives produced class by logistic regression model (glm, family binomial, 'logit' link function) and fitted logistic curve for Western Irish Sea (FU15) throughout 2001-18.

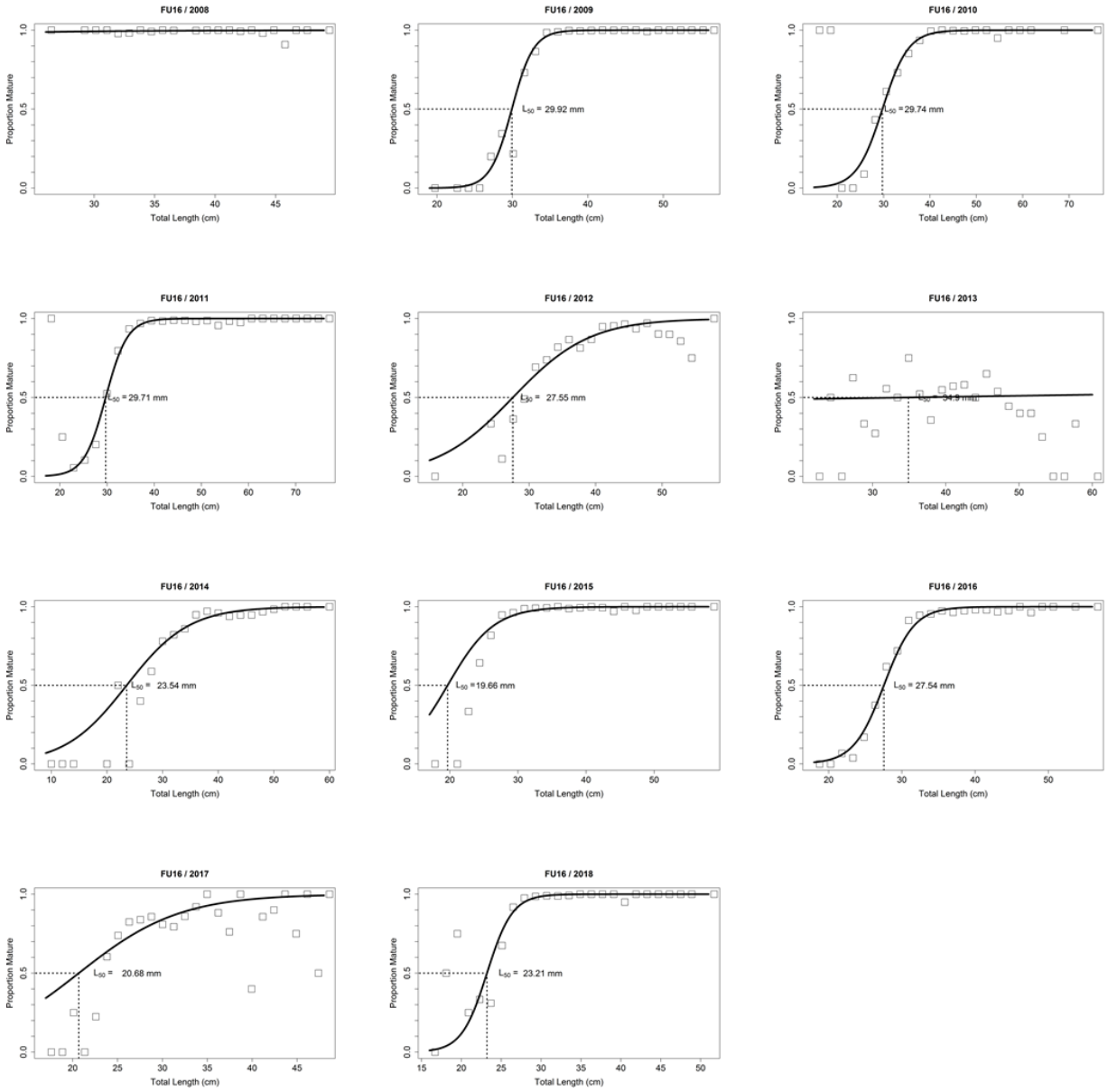

Figure A3.8. L_{50} estimates from maturity ogives produced class by logistic regression model (glm, family binomial, 'logit' link function) and fitted logistic curve for Porcupine Bank (FU16) throughout 2008-18.

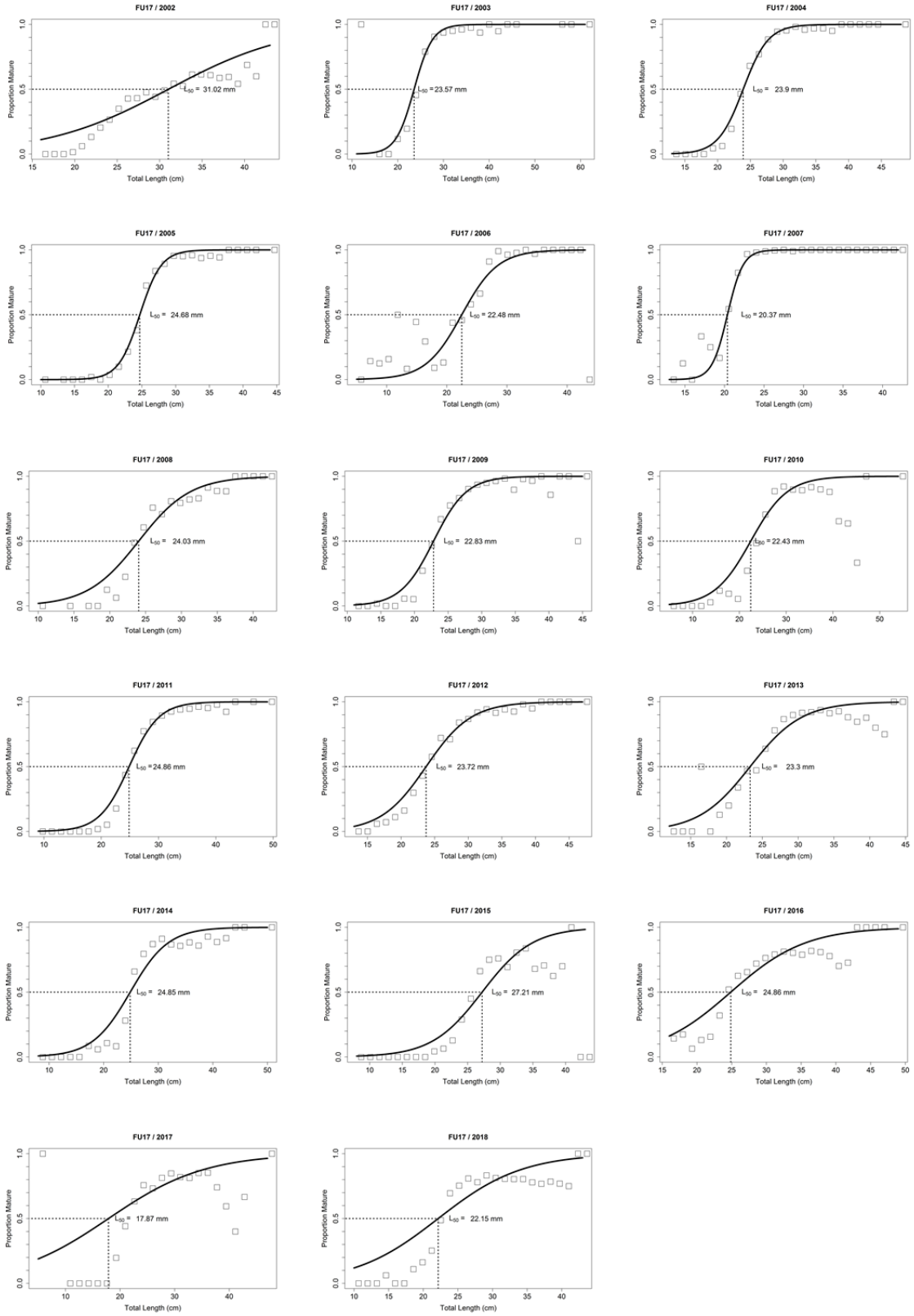

Figure A3.9. L_{50} estimates from maturity ogives produced class by logistic regression model (glm, family binomial, 'logit' link function) and fitted logistic curve for Aran Grounds (FU17) throughout 2002-18.

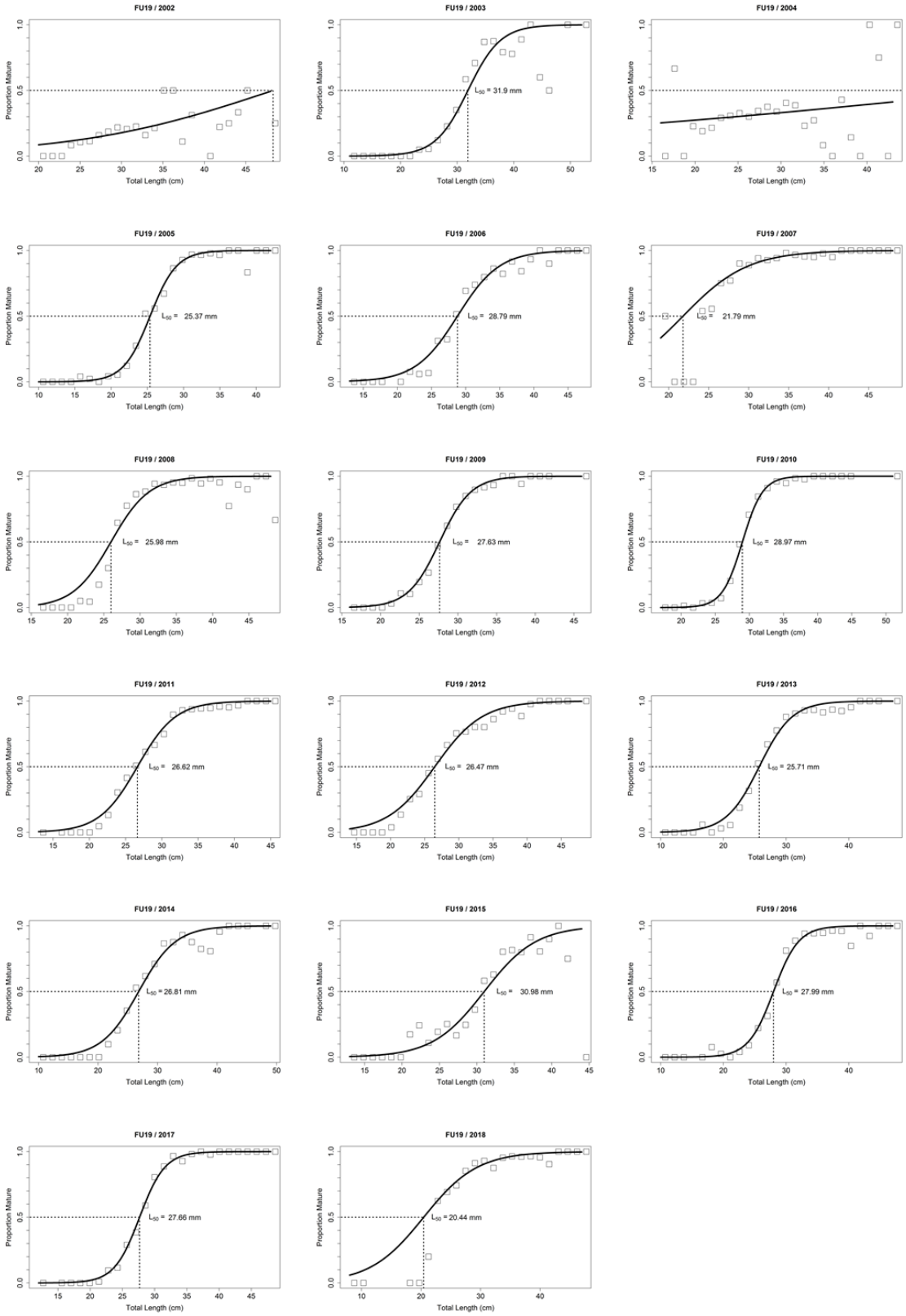

Figure A3.10. L_{50} estimates from maturity ogives produced class by logistic regression model (glm, family binomial, 'logit' link function) and fitted logistic curve for South Coast (FU19) throughout 2002-18.

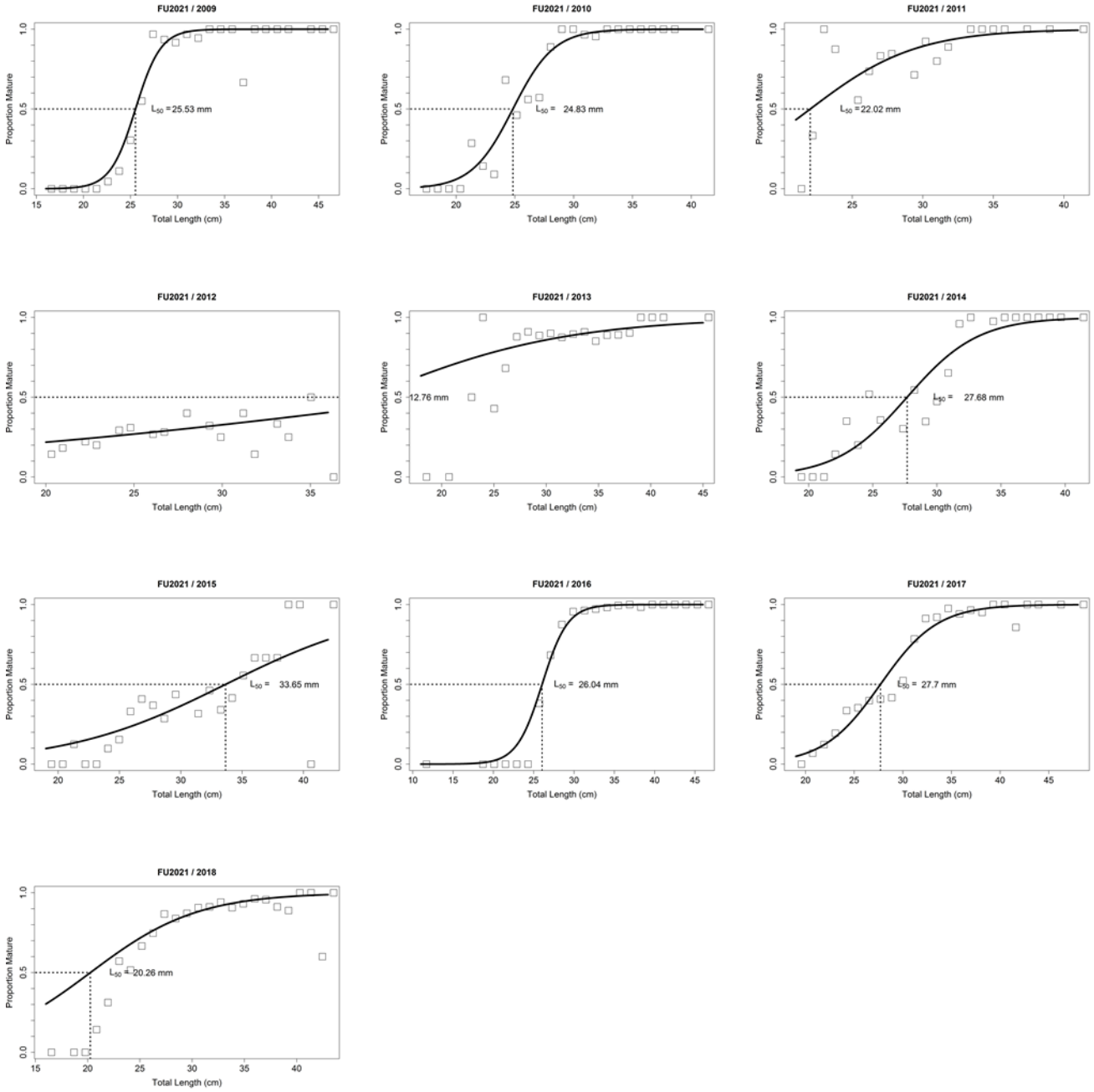

Figure A3.11. L_{50} estimates from maturity ogives produced class by logistic regression model (glm, family binomial, 'logit' link function) and fitted logistic curve for Labadie, Jones and Cockburn Banks (FU2021) throughout 2009-18.

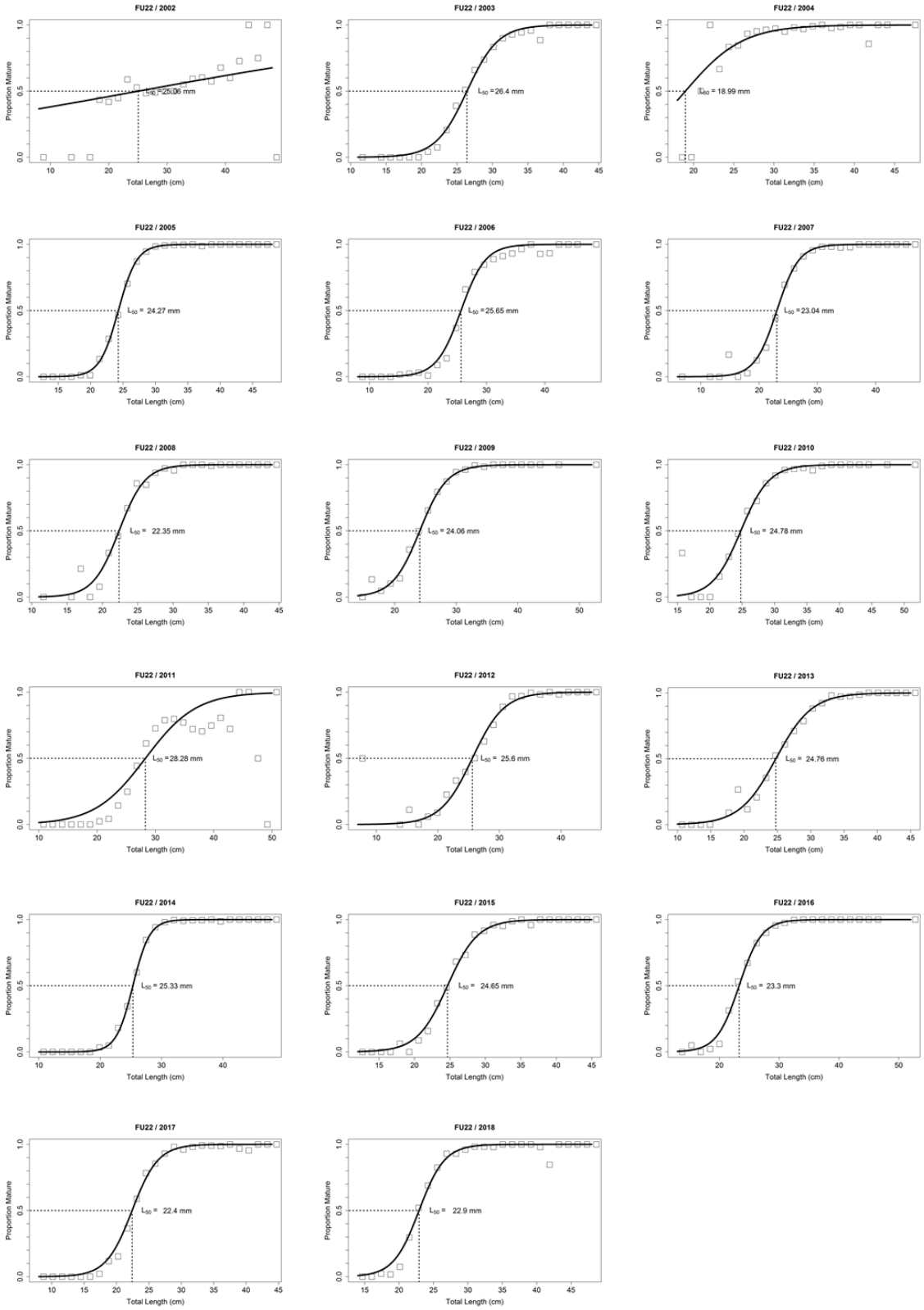

Figure A.3.12. L_{50} estimates from maturity ogives produced class by logistic regression model (glm, family binomial, 'logit' link function) and fitted logistic curve for The Smalls (FU22) throughout 2002-18

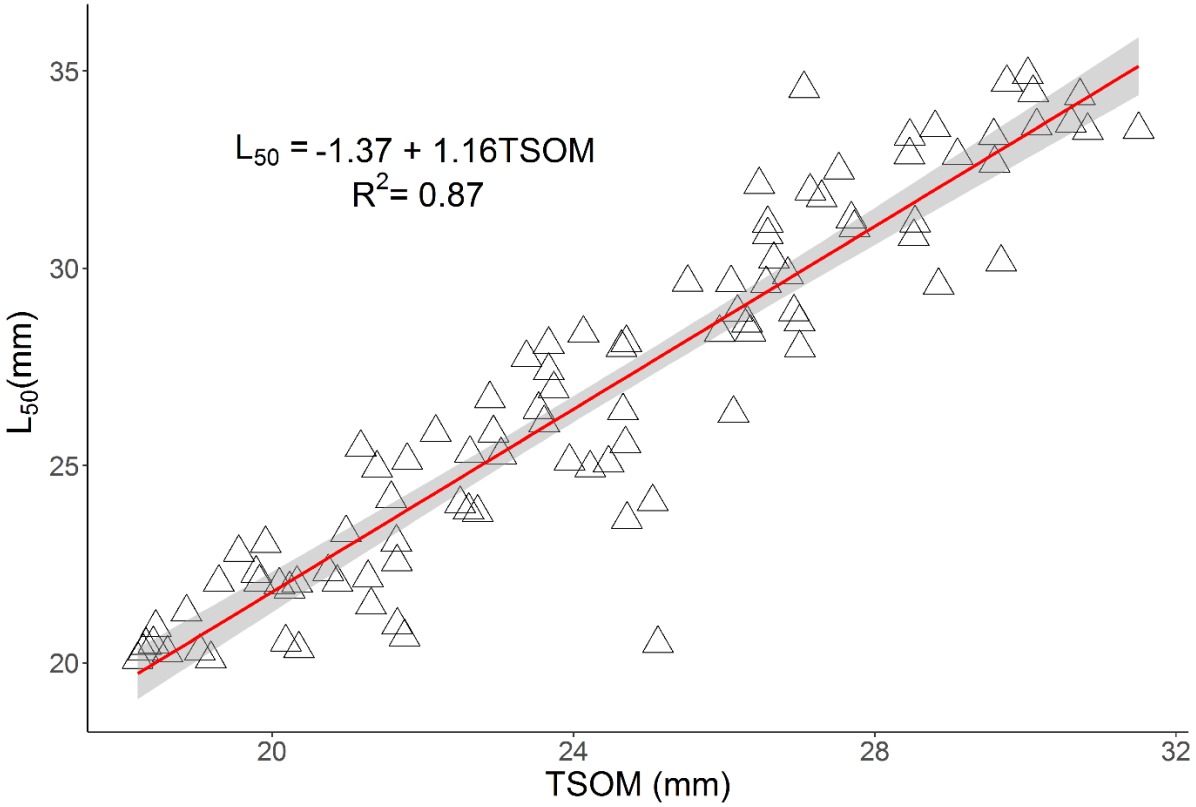

Figure A3.13. Linear regression model showing a significant positive relationship between female *Nephrops* L_{50} (CL, mm) and TSOM (CL, mm) obtained from simulation tests, with annual values per FU presented for Western Irish Sea (FU15), Porcupine Bank (FU16), Aran Grounds (FU17), South Coast (FU19), Labadie, Jones and Cockburn Banks (FU2021) and The Smalls (FU22). 95% confidence interval of the predicted values in grey.

7.3. Appendix 3 – Supplementary Material for Chapter 4

Table A4.1. Sample size (n) of *Nephrops* used for estimation of the size at the onset of maturity using morphometrics (MSOM) i.e. relationships between carapace length and other hard structures in functional management units (FUs): Moray Firth (FU9), Firth of Clyde (FU13), Western Irish Sea (FU15), Aran Grounds (FU17) and The Smalls (FU22)

Sex	FU9	FU13	FU15	FU17		FU22	
	2018	2018	2018	2017	2018	2017	2018
Female	**50**	**27**	**250**	**239**	**250**	**230**	**79**
Male	**45**	**65**	**250**	**217**	**250**	**251**	**160**

Table A4.2. Output of the log-transformed regression model between dimensions of body structures that provided significant MSOM (dependent variable) and inconsistent output concerning type of allometry and relative growth of the body structure to CL after MSOM versus carapace length (independent variable) of male *Nephrops* from Moray Firth (FU9), Firth of Clyde (FU13), Western Irish Sea (FU15), Aran Grounds (FU17) and The Smalls (FU22), as well as MSOM and behaviour of relative growth of body structures dimensions to CL after MSOM..

Ground	Year	Trait	N	r^2	Intercept	Slope			Diagnosis	MSOM	Relative growth after MSOM
						B	2.5% CI	97.5% CI			
FU9	2018	AbW	45	0.97	-0.58	0.97	0.92	1.02	Isometry	35.6	decrease growth rate
FU9	2018	AM	44	0.83	-2.10	0.99	0.85	1.13	Isometry	35.68	decrease growth rate
FU13	2018	AbW	65	0.98	-1.04	1.10	1.06	1.14	Positive allometry	35.04	decrease growth rate
FU13	2018	AM	43	0.81	-4.37	1.66	1.41	1.91	Positive allometry	26.68	decrease growth rate
FU15	2018	ETel	241	1.00	1.06	1.01	1.00	1.02	Positive allometry	26.78	decrease growth rate
FU15	2018	CTel	241	1.00	0.94	1.03	1.02	1.04	Positive allometry	26.6	decrease growth rate
FU15	2018	AbL	242	0.93	0.36	1.07	1.03	1.10	Positive allometry	33.29	decrease growth rate
FU15	2018	AM	234	0.92	-2.76	1.27	1.23	1.32	Positive allometry	25.09	decrease growth rate
FU15	2018	Pl1	233	0.73	-3.59	1.42	1.31	1.53	Positive allometry	25.63	decrease growth rate
FU17	2017	WL2_ML	153	0.96	-0.45	0.99	0.96	1.03	Isometry	30.7	increase growth rate
FU17	2017	AM	125	0.86	-3.92	1.54	1.43	1.65	Positive allometry	20.21	decrease growth rate
FU17	2017	Pl1	126	0.86	-2.39	1.17	1.09	1.25	Positive allometry	19.66	decrease growth rate
FU17	2018	AbL	249	0.76	0.65	0.98	0.91	1.04	Isometry	30.4	decrease growth rate
FU17	2018	CruL	98	0.60	0.64	0.92	0.77	1.07	Isometry	25.54	increase growth rate
FU17	2018	CutL	142	0.73	0.15	1.07	0.96	1.18	Isometry	26	increase growth rate
FU17	2018	CutW	142	0.67	-1.46	1.06	0.94	1.19	Isometry	29.88	increase growth rate
FU17	2018	AM	105	0.68	-4.25	1.62	1.40	1.83	Positive allometry	22.46	decrease growth rate
FU17	2018	Pl1	231	0.71	-2.25	1.11	1.02	1.20	Positive allometry	22.43	decrease growth rate
FU22	2017	ETel	247	0.75	0.97	1.04	0.97	1.12	Isometry	27.64	decrease growth rate
FU22	2017	AbL	247	0.98	0.48	1.04	1.02	1.06	Positive allometry	27.72	decrease growth rate
FU22	2017	WL2_MW	211	0.88	-3.54	1.28	1.21	1.34	Positive allometry	29.34	decrease growth rate
FU22	2017	AM	42	0.93	-4.28	1.65	1.50	1.79	Positive allometry	27	decrease growth rate
FU22	2017	Pl1	41	0.94	-2.92	1.34	1.22	1.45	Positive allometry	25.1	decrease growth rate
FU22	2018	WL2_MW	107	0.93	-3.16	1.17	1.11	1.24	Positive allometry	29.32	decrease growth rate
FU22	2018	AM	158	0.89	-3.88	1.52	1.44	1.61	Positive allometry	27.07	decrease growth rate
FU22	2018	Pl1	158	0.90	-2.35	1.16	1.10	1.22	Positive allometry	27.32	decrease growth rate

Table A4.3. Output of the log-transformed regression model between dimensions of body structures that provided significant MSOM (dependent variable) and inconsistent output concerning type of allometry and relative growth of the body structure to CL after MSOM versus carapace length (independent variable) of female *Nephrops* from Moray Firth (FU9), Firth of Clyde (FU13), Western Irish Sea (FU15), Aran Grounds (FU17) and The Smalls (FU22), as well as MSOM and behaviour of relative growth of body structures dimensions to CL after MSOM.

Ground	Year	Trait	N	r^2	Intercept	Slope			Diagnosis	MSOM	Relative growth after MSOM
						b	2.5% CI	97.5% CI			
FU9	2018	CTel	50	0.99	1.02	1.01	0.98	1.04	Isometry	25.46	decrease relative growth
FU9	2018	CutW	40	0.95	-1.34	1.02	0.94	1.10	Isometry	37.18	increase relative growth
FU13	2018	WL2_ML	27	0.89	-0.78	1.08	0.91	1.25	Isometry	19.69	decrease relative growth
FU15	2018	BL	250	0.99	1.07	1.03	1.01	1.05	Positive allometry	21.89	decrease relative growth
FU15	2018	ETel	250	0.99	1.06	1.02	1.00	1.03	Positive allometry	22.07	decrease relative growth
FU15	2018	CTel	250	0.99	0.98	1.02	1.01	1.03	Positive allometry	23.56	decrease relative growth
FU15	2018	AbL	250	0.97	0.48	1.03	1.01	1.05	Positive allometry	21.81	decrease relative growth
FU15	2018	WL2_CL	250	0.88	-1.29	0.96	0.91	1.01	Isometry	33.4	increase relative growth
FU17	2017	AbL	239	0.84	0.37	1.07	1.01	1.13	Positive allometry	25.29	decrease relative growth
FU17	2017	CutD	118	0.88	-1.89	1.06	0.99	1.14	Isometry	34.83	increase relative growth
FU17	2018	CW	250	0.88	-0.83	1.04	1.00	1.09	Isometry	27.06	increase relative growth
FU17	2018	AbL	250	0.95	0.50	1.03	1.00	1.05	Isometry	26.03	decrease relative growth
FU17	2018	WL2_CL	250	0.77	-1.64	1.02	0.94	1.10	Isometry	22.54	increase relative growth
FU22	2017	AbW	230	0.97	-1.29	1.21	1.18	1.23	Positive allometry	32.25	decrease relative growth
FU22	2018	CruL	10	0.96	-0.57	1.28	1.07	1.49	Positive allometry	22.26	decrease relative growth
FU22	2018	CruW	10	0.90	-2.33	1.38	1.03	1.73	Positive allometry	22.64	decrease relative growth
FU22	2018	CruD	10	0.79	-3.56	1.64	1.01	2.28	Positive allometry	23.71	decrease relative growth

Table A4.4. Summary of linear model fit between annual estimates of male *Nephrops*' morphometrical size at the onset of maturity (MSOM) based on the pair carapace length-appendix masculina and population density across all functional management units (FUs) considered in the study.

Variable	Coefficient	Std. Error	t-value	p-value
Intercept	31.97	4.63	6.91	0.001
Density	-10.26	7.78	-1.32	0.24
R-square	0.26	Adjusted R-squared	0.11	
F-statistic	1.737	Degrees of freedom	5	
p-value	0.24	Residuals std. error	4.60	

Table A4.5. Summary of linear model fit between annual estimates of male *Nephrops*' morphometrical size at the onset of maturity (MSOM) based on the pair carapace length-first pleopod and population density across all functional management units (FUs) considered in the study.

Variable	Coefficient	Std. Error	t-value	p-value
Intercept	30.03	5.48	5.48	0.001
Density	-6.33	9.21	-0.69	0.52
R-square	0.09	Adjusted R-squared	-0.10	
F-statistic	0.47	Degrees of freedom	5	
p-value	0.52	Residuals std. error	5.44	

Table A4.6. Summary of linear model fit between annual estimates of male *Nephrops*' morphometrical size at the onset of maturity (MSOM) based on the pair carapace length-crusher propodus length and population density across all functional management units (FUs) considered in the study.

Variable	Coefficient	Std. Error	t-value	p-value
Intercept	35.74	4.85	7.36	0.002
Density	-10.37	7.62	-1.36	0.25
R-square	0.32	Adjusted R-squared	0.15	
F-statistic	1.85	Degrees of freedom	4	
p-value	0.25	Residuals std. error	3.52	

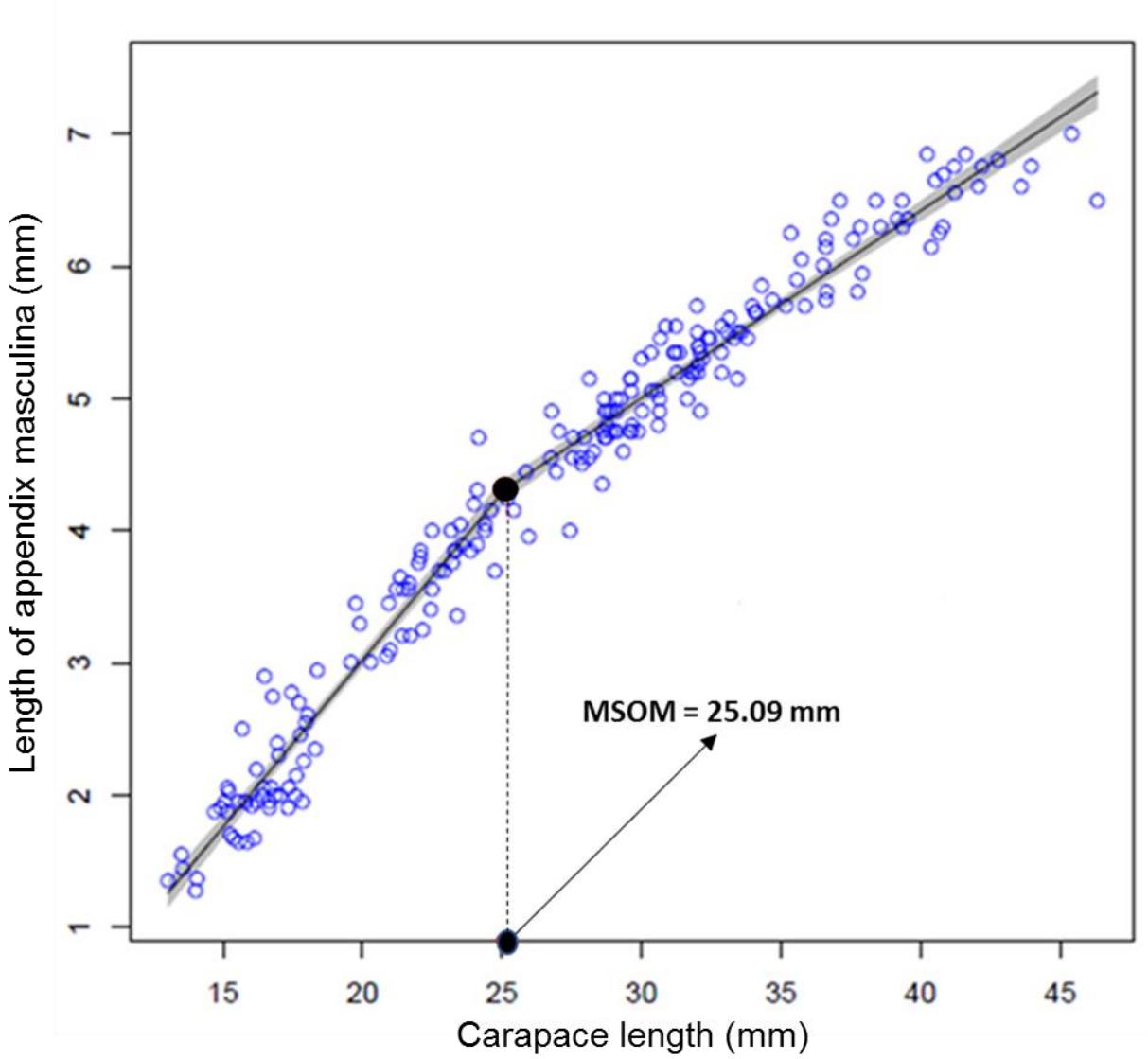

Figure A4.1. Segmented regression plot of the morphometric pair CL-Appendix masculina for male *Nephrops* at Western Irish Sea (FU15) in 2018, with indication of the MSOM estimate (CL, mm) for this FU.

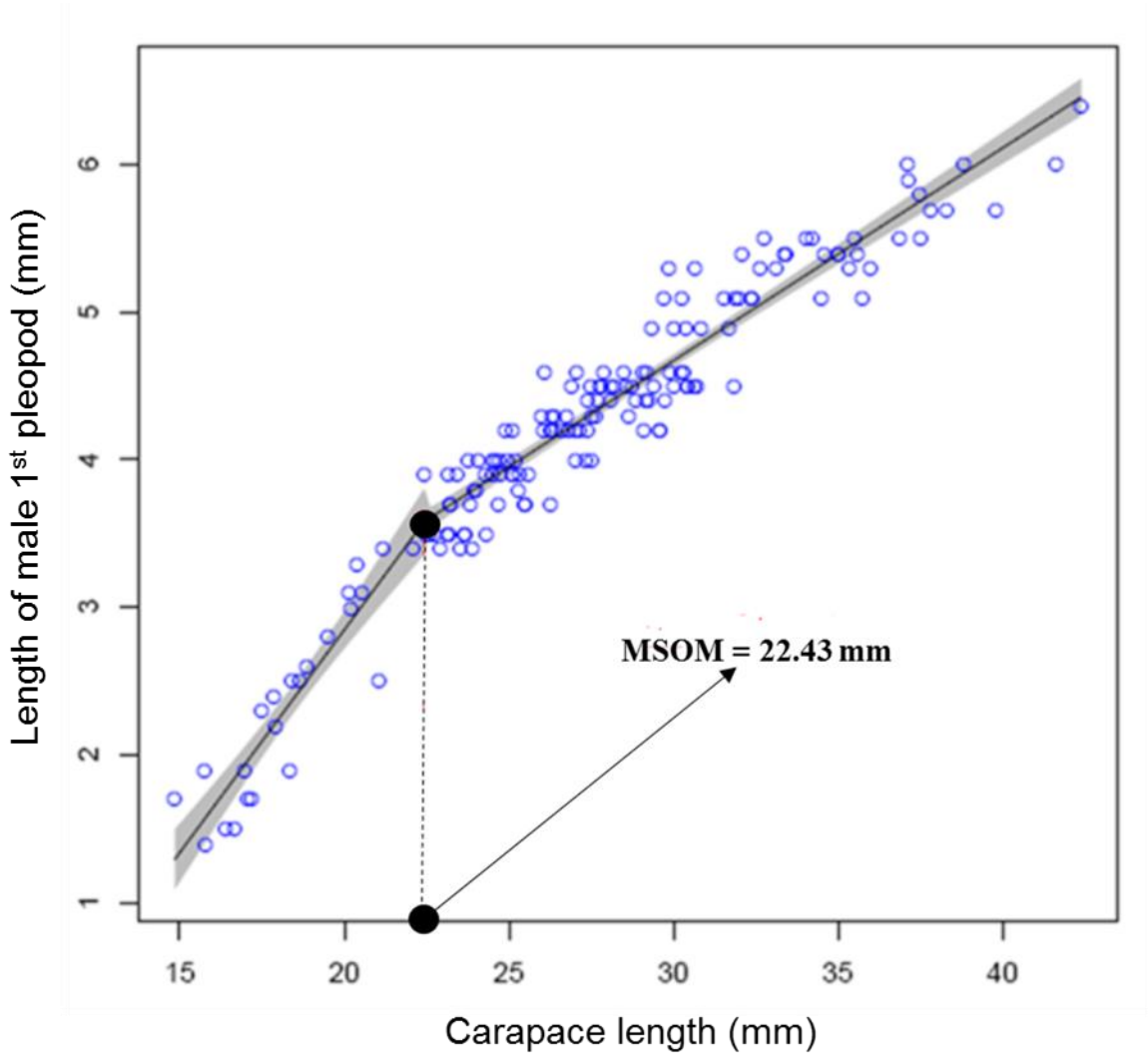

Figure A4.2. Segmented regression plot of the morphometric pair CL-Male 1st pleopod for male *Nephrops* at Aran Grounds (FU17) in 2018, with indication of the MSOM estimate (CL, mm) for this FU.

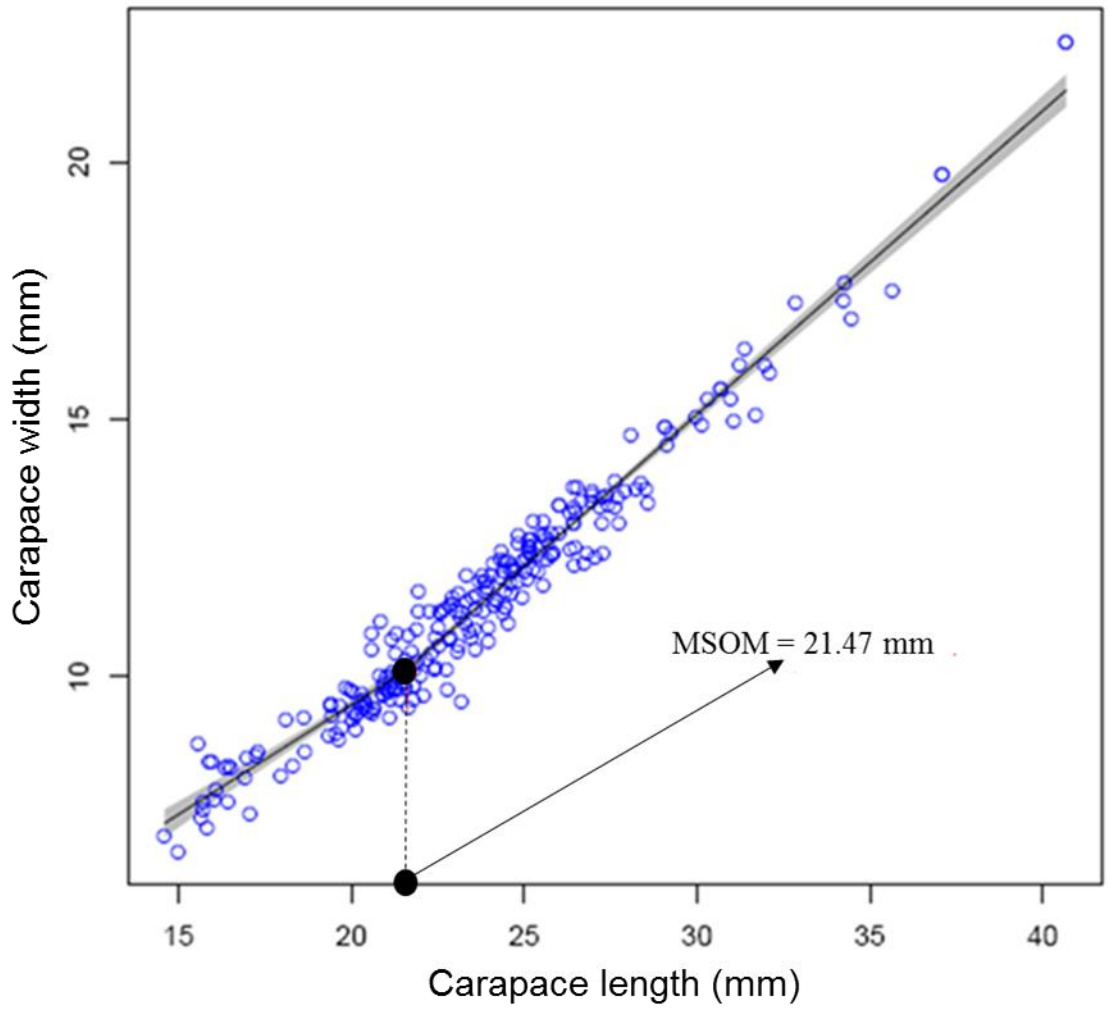

Figure A4.3. Segmented regression plot of the morphometric pair CL-Carapace width for female *Nephrops* at Western Irish Sea (FU15) in 2018, with indication of the MSOM estimate (CL, mm) for this FU.

Code and other material

The code and other files concerning Chapter 4 and output not included herein will be available at https://github.com/Caesar-Santana/PhD_Thesis_Scripts.git.