Fisheries & Conservation Science

Investigating the abundance, distribution and habitat use of juvenile *Cancer pagurus* (L.) of the intertidal zone around Anglesey and Llŷn Peninsula, North Wales (UK).

PRIFYSGOL BANGOR UNIVERSITY

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Thesis submitted to the School of Ocean Sciences, University of Wales, Bangor, in partial fulfilment of the requirement to the degree of:
MSc Marine Biology

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To be cited as follows: Heraghty, N. (2013). Investigating the abundance, distribution and habitat use of juvenile *Cancer pagurus* (L.) of the intertidal zone around Anglesey and Llŷn Peninsula, North Wales (UK). MSc thesis, Bangor University, Fisheries & Conservation report No. 29, Pp.75

EUROPEAN FISHERIES FUND

DECLARATION

This work has not previously been accepted in substance for any degree and is not being currently submitted in candidature for any degree.

This dissertation is being submitted in partial fulfilment of the requirement of the M.Sc. in Marine Biology.

This thesis is the result of my own investigations, except where otherwise stated. Where correction services have been used, the extent and nature of the correction is clearly marked in a footnote(s).

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Acknowledgments
I would like to thank Professor Michel Kaiser and Dr Jodie Haig for their help, guidance and support throughout the organisation, undertaking and writing of this project. I would like to thank members of the European Fisheries fund team for their time and help when conducting the mark and recapture study within this project. Thank you to Dr Dei Huws for his advice with laboratory techniques. Thank you to Berwyn Roberts for advice on animal husbandry during captivity of the crabs. Thank you to Michael Hayle for his help, support and organisation of the project. Finally thank you to my family and friends who have continually supported and encouraged me throughout the project.

Abundance, distribution and habitat use of juvenile *Cancer pagurus* (L.) in the intertidal zone of Anglesey and the Llŷn Peninsula, North Wales (UK)

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Abstract

A study was conducted to collect information on the abundance, distribution and habitat use of juvenile *Cancer pagurus* on the Isle of Anglesey and Llŷn peninsula north Wales (UK). The abundance data is intended to be collated with a time series to create a recruitment index for this heavily exploited species. This is necessary for science based management plans to be formed to maintain the sustainability of the fishery. Currently there is limited information on the life history of this species, this study provided information of sites which host a population of juvenile *C. pagurus* and investigated biotic and abiotic factors which may influence the presence of the species. Boulders were found to be an indicative factor which may influence the species as it is able to shelter the individuals from predation and environmental parameters that are detrimental to them. A mark and recapture study was also conducted providing a total population estimate of = 9442 (± 146.65 individuals). Death and emigration rates = -1.11 with birth and immigration rates = 0.955. The hepatopancreas wet weight of individuals from each site was recorded as a condition factor to investigate the nutritional state at each site. No significant difference was found between sites (ANOVA, F_3 = 0.702, P = 0.555). Inter annual differences between abundance and carapace width was identified between the data collected in 2012 and 2013 (ANOVA, $F_{1,32}$ = 21.204, P <0.01). This difference was attributed to higher average temperatures during spring of 2012 which would have reduced the intermoult period and influence early settlement into the intertidal area.

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Abbreviations

Anosim	Analysis of similarities
Anova	Analysis of Variance
C.D	Chart Datum
CHH	Crustacean hyperglycemic hormone
CPUE	Catch per unit effort
C.W	Carapace width
FAO	Food and agricultural organization of the UN
GIS	Geographic information system
GLM	General linear model
GPS	Global positioning system
ICES	International council for the exploration of the sea
LPUE	Landings per unit effort
MDS	Multidimensional scaling
S.E	Standard error
SRR	Stock to recruit relationship

1.0. Introduction

The crustacean *Cancer pagurus,* commonly known as both the European edible crab or brown crab, is an important crustacean to the global fishing industry and is one of the most important species in English and Welsh fisheries. Despite this species being heavily fished little information has been collected regarding its ecology and life history leaving it vulnerable to over exploitation and stock loss. Understanding each life history stage of an exploited species helps understand the reproductive potential of an adult and the recruitment potential of the next generation known as the stock to recruit relationship (SRR) allowing sustainable harvests to be made (Wahle 2003). Research into the ecology and distribution at the juvenile stage of *C. pagurus* has been neglected and this study was conducted to fill in gaps about the distribution, abundance, and habitat use of juvenile brown crabs, *Cancer pagurus*, within the intertidal area of rocky shores around the North Wales coastline. This research builds on from a study conducted by Dickinson (2012) and will highlight sites where juvenile *C. pagurus* are present, provide information on abiotic and biotic factors that promote survival and growth with the intention of describing nursery habitats. In subsequent years abundance data will be combined to produce a juvenile recruitment index, to give the number of *C. pagurus* that can be sustainably taken from the adult population and allow fisheries managers to make informed decisions regarding management strategies.

1.1 Taxonomy and Distribution

Cancer pagurus belongs to the Cancridae family (Decapoda: Crustacea) (Neal & Wilson 2008). It distribution spans throughout the North Sea, the English Channel and the coast of Portugal (Anosov 2000). Commercial exploitation takes place around Britain, Ireland, Norway, Sweden and France (Tully *et al* 2006). The adults have a benthic existence and are found from the intertidal zone to depths of 100m (Neal & Wilson 2008). Recruitment into the adult population takes place at 4 to 6 years of age and life expectancy is about 15 years (Tully *et al* 2006).

1.2. Crab fisheries

In the UK fishing for *C. pagurus* occurs mainly between April and November and is influenced by environmental factors and crab behaviour, such as moulting and migration (Edwards 1978). The crabs are fished using baited traps known as pots (Figure 1), which are basically a weighted frame covered with mesh. Around the edge of the pot are openings, known as eyes, these allow the crab to enter but are funnelled preventing their escape. Bait, which is used to entice the crabs into the pot, is usually connected to the frame in the middle of the pot. Choice of bait varies but fresh fish is thought to be more attractive than synthetic bait (Edwards 1978). Many pots are connected together on a long main rope known as a string. This is then cast into the water, at either end of the string is an anchor used to secure the pots to the sea floor. The pots are set during the day and left overnight, as the crabs are known to actively forage at night, and ideally hauled the next morning (Edwards 1978).

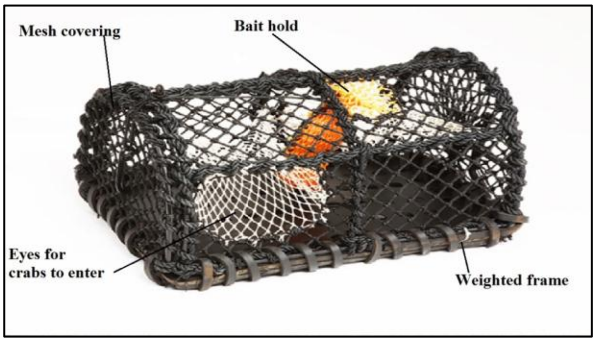


Figure 1. A modern parlour pot with plastic mesh describing the basic baited trap used to catch *C. pagurus* in the sea (Medley pots 2013)

1.2.1. Importance of *C. pagurus* in the fishery

Cancer pagurus is important to global crustacean fisheries, FAO statistics report that global landings of *C. pagurus* have been steadily increasing from 1950 to 2011 (Figure 2). The UK fisheries contribute a large proportion to global catches, and within England and Wales *C. pagurus* fishing is one of the most important fisheries (FAO 2013). In 2011 around 12000 tonnes were landed in England and Wales with an estimated worth of €19 million at first sale, equating to around £16 million (ICES 2011). Since 1978 landings from the Irish Sea have been increasing and more catches have been landed into Welsh ports (Edwards 1978).

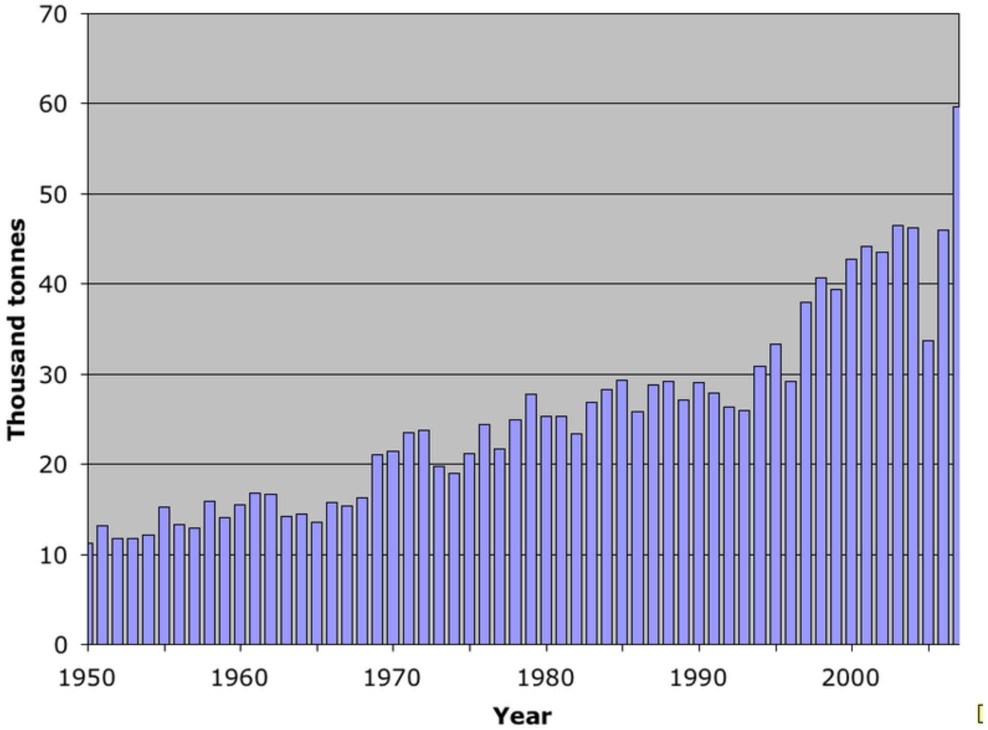


Figure 2. Global annual landings (Thousand tonnes) of *C. pagurus* from 1950 – 2007 as reported by ICES (2008).

Although no stock collapse has been reported for the Irish Sea population. Fisheries managers have raised concerns that there is potential for overfishing as landings per unit effort (LPUE) are declining (Tully *et al* 2006), and a reduction in catch per unit effort (CPUE) has been suggested to allow sustainability of the stock (Bannister 2009).

1.2.1. Threats to Irish Sea stocks and current management

The main threat to crab stocks is over fishing, as there is little regulation on the industry. The crabs are often caught as bycatch by the lobster fisheries and actively caught as bait for whelk fishing. The numbers of crabs taken for these industries are not reported so impacts on the stocks are unknown (Tully *et al* 2006).

Current management for *C. pagurus* stocks includes the prevention of landing individuals below a minimum landing size and oviparous females (egg carrying females). On the west coast of the UK there are two minimum landing sizes, which are dependent on the area. Between 48- 56^{o}N there is a minimum landing size of 130 mm, north of 56^{o} minimum landing size is 140mm. No restrictions are placed on the time of year or area fishing takes place. CPUE is unregulated and the size and number of traps deployed by a commercial fishing vessel is unchecked (Tully *et al* 2006).

1.3. Reproduction and larval stage

Like most crustaceans *C. pagurus* has a complex life history that includes a benthic existence and a dispersive planktonic larval stage (Pallas 2006). Size at maturation is subject to environmental conditions such as salinity and temperature. In areas with higher water temperature crabs mature earlier and at a smaller size due to shorter intermoult periods. Crabs that live in areas of lower salinity also mature at a smaller size although there are tolerance limits to lower salinity levels (Fisher 1999).

Copulation occurs during ecydis of the female (shedding of the shell). After copulation there is a delay of between 3 – 15 months before the development of the female gonads. This is due to the depleted energy levels experienced by the female after moulting (Warner 1977). During this time females have been found to migrate long distances, possibly to release their young in an optimum habitat. In the North Sea they have been found to move north, ensuring that upon release the larvae are carried south with the sea currents to the parent population (Nicholas *et al* 1982 & Lindley 1987)). In the English Channel the females travel west where the substrate changes to a sandy gravel mix (Nicholas *et al* 1982). Here, the female can successfully attach her eggs to the pleopods (small hair on the legs of the female). Laboratory experiments conducted by Edwards (1966) revealed that females require sandy or shingly substrata to enhance the attachment of the eggs to the pleopods. The substratum allows the female to dig a pit in which to release her eggs preventing them from being lost to the currents before moving the abdomen in an up and down motion allowing the eggs to attach to the pleopodal setae (Edwards 1978). It also allows the larvae to travel east with the current back to the parent population.

The female will brood each batch of eggs for a period of 7 to 8 months before the larvae hatch and are released into the plankton (Edwards 1978). The larvae have a planktotrophic existence remaining in the water column for approximately 60 days (Weiss *et al* 2009). During this time the larvae will develop through 5 zoeal stages and 1 megalopa (post larval) stage before settling into the intertidal habitat and metamorphosing into a juvenile (Nicholas *et al* 1982). Upon settling, juvenile *C. pagurus* are small and highly vulnerable, therefore settling into an area that enhances their post settlement survival might be advantageous to them (Wahle 2003).

1.3.1. Larval Settlement

The settlement and survival at the megalopae stage strongly influences the presence of juveniles in an area (Moksnes 2002). Settlement into a suitable area will promote survival and growth of the individual especially as migration after settlement is thought to be limited in small juveniles. This is because locomotion is difficult over the habitat and they are highly vulnerable to predation (Etherington & Eggleston 2000).

The habitat that the larvae settle in is species specific and based on different abiotic and biotic factors, such as the presence of conspecifics, absence of predators or chemical cues of the substrata (Montfrans *et al* 2003). Megalopa of the shore crab, *Carcinus maenas* and the blue crab, *Callinectes sapidus*, have been shown to actively settle into habitats described as structurally complex (Etherington & Eggleston 2000). These habitats include mussel beds, rocky shores, eel beds and macro algae (Moksnes 2002). They are effective habitat for juveniles as they offer protection from predators and have a higher availability of food (Stoner *et al* 2003).

1.4. Nursery Habitats

Nursery habitats have been described as areas where juveniles occur at higher densities, avoid predation more successfully or grow faster than in other habitats (Pallas *et al* 2006). Also an area used by juveniles separate from adults with a direct migration to the adult population. As no strict definition has been produced many areas have been named nurseries due to a high level of juvenile abundance, however after further analysis successful recruitment to the adult population is found to be low resulting in the habitat being unimportant for the growth of the population. Therefore it is uncertain if these areas fulfil a nursery function or if high abundances are a result of other factors such as high larval influx (Beck *et al* 2001). A nursery habitat important in one area may not be important in another location due to local abiotic and biotic factors, therefore nursery habitats should be investigated on a localised scale (Beck *et al* 2001).

Juvenile *C. pagurus* are thought to settle into the intertidal zone until they are around 70 mm in size where they begin to move subtidal and join the adult population (Bennett & Pawson 1995). Size class segregation may occur due to ontogenetic shift in habitat use as the individual grows suggesting larger individuals may move down the shore towards the adult population (Wahle 2003). Cannibalism and antagonistic behaviour between conspecifics is another reason for size class segregation both of which behaviours have been seen in *C. pagurus* (Moksnes 2004 & Amaral *et al* 2009).

1.5. Hepatopancreas as a measure of fitness

The nutritional state of individuals may indicate habitat suitability. The weight of a fish compared to its length, known as a condition factor, is often used in studies to give an indirect energy status, as it implies the 'fitness' of an individual. A hepatosomatic index, the wet or dry weight of the liver, gives a direct indication of energy status and can be used to predict growth rate in many fish species, however, the process does require dissection which can be impractical to many studies (Chellappa *et al* 1995).

Crustaceans have a large organ involved in the digestion and storage of metabolic reserves known as the hepatopancreas (Warner 1977) this can be weighed similarly to give a hepatosomatic index (Chellappa 1995). The organ is located behind the anterior margins of the carapace and extends over the anterior ends of the branchial chambers ending between the muscles of the leg bases. When feeding the crab places the food to its mandibles and breaks it down. The food is then passed along the oesophagus to a stomach, which is split into two regions, the cardiac stomach, where digestive juices from the hepatopancreas are introduced to the food, (Walter 1960) and pyloric stomach (where the food is sorted before being passed to the mid gut and is absorbed by the hepatopancreas) (Lockwood 1968).

The nutritional state of the hepatopancreas is affected by the moult stage of the crab. Shortly before ecydis an increase of water into the body occurs and is stored in the hepatopancreas. During this time glycogen is moved from the organ to the epidermal cells under the new cuticle in preparation for an increase in energy required for the formation of chitin when the new shells harden (Lockwood 1968).

1.6. Diet of *C. pagurus*

Adult crabs have been described as nocturnal predators to reduce the risk of falling prey to fish and seals. At the juvenile stage the crabs are active predators and will prey on a variety of crustaceans including Carcinus maenas, *Porcellana platychelas* and *Mytilus edulis* (Lawton 1989 and Mascaro & Seed 2001). The crabs stalk their prey until pouncing, trapping the prey under their abdomen and crushing it with the chelae (Mascaro & Seed 2001). Juveniles are limited in their predation abilities by the strength of their chelae and will take crustaceans of a slightly smaller size than their capabilities, to be more energy efficient and to limit damage to their chelae during the crushing of their prey which may reduce feeding ability later (Mascaro & Seed 2001).

1.7. Mortality

Knowledge of both the natural and fishing mortality rate throughout all life stages to understand when selective pressures are most intense (Gosselin 1997). Disregard of this knowledge can impact the biology (reducing size at maturity), productivity and genetic diversity of the stock (Begg *et al* 1999, Gosselin 1997). Management plans will be more effective if recruitment can be predicted should a stock need to be rebuilt (Begg *et al*

1999). Causes of mortality such as predation, disease, intra and interspecific competition should also be investigated to ensure that management strategies are effective when implemented (Gosselin 1997).

In many invertebrate juveniles mortality is extremely high upon first settlement but has a sharp decline after the first day, changes in anatomy and behaviour may cause this reduction. As the individual grows they become less vulnerable to predation and physical constraints so rate of mortality is reduced (Gosselin 1997).

Methods for understanding mortality for many benthic invertebrates include mapping the fate of individuals by recording the presence and absence of sessile individuals and devising a survivorship curve. Mark and recapture and cohort measuring are becoming more prominently used, however, these are difficult to conduct on motile species and do not reveal the cause of mortality (Gosselin 1997).

Dickinson (2012) conducted a study with the main intention of formulating a methodology that would measure the abundance of juvenile *C. pagurus* found in the intertidal zone. The same methodology could then be replicated over subsequent years to build up a data set that could be used to form a juvenile recruitment index. Environmental parameters were also investigated to find which abiotic and biotic factors may be important to the presence and survival of the crabs. A positive relationship was found between boulder substratum and the abundance of juvenile *C. pagurus* indicating the preference for a structurally complex habitat upon settlement.

1.8. Hypotheses & Objectives

In order to fill knowledge gaps regarding the ecology and life history of *C. pagurus* and to continue on from Dickinson (2012) a study was conducted to investigate the abundance, distribution and habitat use of juvenile *C. pagurus* around North Wales with the aim of concluding the following hypotheses and completing the following objectives.

Hypotheses

H_1: Structurally complex shores will have a higher abundance of juvenile *C. pagurus* than less structurally complex habitats.

H_2: Segregation in size classes within the habitat will be observed with individuals of a larger carapace width living lower down the shore.

H_3: Biotic and abiotic characteristics of the habitat will have an impact on the abundance of juvenile *C. pagurus*.

H_4: Individuals with a higher hepatosomatic index will be found in habitats with a higher juvenile *C. pagurus* abundance.

Objectives

1. Assess the abundance of juvenile *C. pagurus* and identify suitable habitats in the intertidal area of the north Welsh coastline using timed searches in a measured area.
2. Assess the distribution of juvenile *C. pagurus* using carapace width measurements and their position on the shore.
3. Assess the habitat use of juvenile *C. pagurus* using a mark and recapture study to observe the movement patterns of the crabs over a tidal cycle.
4. Determine the total abundance of juvenile *C. pagurus* at one site using a mark and recapture study.
5. Assess the immigration and emigration of juvenile *C. pagurus* into a site using a mark and recapture study.

6. Assess the community composition at each site to identify biotic factors that may influence the presence of juvenile *C. pagurus*.
7. Assess the physical structure of the shore to determine its complexity and identify abiotic factors that may be important to the presence of juvenile *C. pagurus*.
8. Measure the weight of the hepatopancreas from different size classes of juvenile *C. pagurus* from all sites to give a condition factor of the individual and infer which habitats promote growth most successfully.

2.0 Material and Methods

2.1 Site selection

Sites suitable for sampling were found using biotope maps issued by Natural Resources Wales. Using ArcGIS, rocky areas around Anglesey and North Wales (ranging from the Llŷn Peninsula to the great Orme) were highlighted. Areas deemed suitable from the biotope maps were assessed for accessibility using Google Earth and Landranger ordnance survey maps 114 and 123. Areas deemed suitable (Appendix A) were visited during June 2013 when tides were <1.5m C.D to check accessibility and determine the presence of juvenile *C. pagurus*.

Accessible shores were surveyed in a random pattern for the presence or absence of *C. pagurus*. This involved lifting boulders, rocks and moving seaweed. Shores inhabited by *C. pagurus* were later revisited for sampling (Figure 3).

Figure 3. Survey sites with shore names sampled during June and July 2013 map adapted from google maps 2013.

2.2 Abundance data for *C. pagurus*

All survey sites were sampled 3 times throughout June and July 2013, when tidal height was <1.5m C.D. The abundance of *C. pagurus* on each shore was determined using 6 timed searches within a measured area, defined using nylon wire (1.5m long) tied to a boulder. Each search area was spaced 10m apart and positioned at the centre of the mid and low shore which were defined using biota as indicators (table 2). The *Ascophyllum nodosum* zone was not surveyed as results from Dickinson (2012) found the abundance of juvenile *C. pagurus* to be negligible.

Each measured area was actively searched over a 15-minute period, this involved turning boulders and moving seaweed to locate the crabs. The carapace width of all individuals found was recorded to the nearest mm.

Table 1. Description of indicator species used for each zone to identify the shore height in the field.

Shore height	**Biological zone**	**Indicator species**	**Algal zone category for this study**
High	Littoral	*Barnacles, Pelvetia canaliculata, Fucus spiralis, Ascophyllum nodosum*	*Ascophyllum nodosum*
Middle	Eulittoral	*Fucus vesiculosus, Fucus serratus*	*Fucoid*
Low	Sublittoral	*Laminaria digitata, Palmaria palmate, Chodrus crispus*	*Laminaria*

2.3 Recording environmental data

Within each search area a $0.25m^2$ quadrat was used to measure the biotic and abiotic factors present. The parameters measured included the percentage cover of algal species present, barnacle and substratum coverage and an abundance count of other species present (Appendix Table B1). During the second sampling period, if possible, a sediment sample was collected and taken back to the laboratory for particle size analysis. Salinity measurements were recorded in the field by digging a small pit and allowing it to fill with water, this water was analysed using a hand held refractometer.

2.3.1 Sediment particle analysis

The wet weight of each sediment sample collected was recorded then dried in an oven at 100^{o}c until completely dry. A subsample of 25g was removed from the dried sample and added to a solution of water (250ml) and hexaphosphate (10ml) to remove salt crystals and left for a period of 12 hours. The sample was then emptied into a meshed sieve with a 63μm aperture and placed back in the oven at 100^{o}c until dry. The treated sample was sieved through Wentworth sieves of decreasing aperture sizes (Appendix table B2) using an industry standard sieve shaker. The weight of sediment collected in each sieve was weighed (+/- 0.01g) and recorded. A gradistat program was used to give the percentage of sediment type per sample. This sediment data was incorporated with the environmental data recorded in the field and analysed using multivariate methods in PRIMER (section 2.5.3).

2.4 Mark and recapture – Laboratory tag retention study

Prior to a mark and recapture study to investigate the distribution and habitat use of juvenile *C. pagurus* in the field a laboratory-based preliminary study was conducted. This preliminary study tested the resilience of the tags and identified any detrimental impacts potentially imposed on tagged individuals.

A total of 20 crabs were collected from the Menai Bridge site during July 2013 and transported to Bangor University Ocean Sciences laboratory in Menai Bridge. This site was chosen due to a high abundance of juvenile *C. pagurus* observed during the searches performed earlier in the month and allowed short transport time reducing stress to the individuals. Upon arrival into the laboratory the crabs were placed into separate compartments within one large tank to avoid antagonistic behaviour of larger *C. pagurus* towards smaller conspecifics which have been observed in previous laboratory studies

(Amaral *et al* 2009). It also allowed for accurate observations of each crab. The temperature of the tank was regulated between 17- 19 ^{o}c, seawater was sourced from the Menai Strait and filtered before being introduced to the tanks. Fresh mussel meat, removed from the shell, was provided daily for each crab. The crabs were allowed to acclimatise for one week before the tag retention study took place. During this time 5 crabs died leaving 15 for the laboratory experiment. The remaining crabs were split into 2 groups, a tagged group containing 7 and control group containing 8 individuals.

A representation of size classes was assigned to each group to allow for a more detailed comparison between the 2 conditions. Crabs could not be conveniently assigned groups due to their sex, as there was not an even representation. To apply the tags the carapace area to be marked was dried with a paper towel and a small amount of Locktight glue was applied, the tag was placed on the glue and held into place using a small plastic rod. The crab was left in a dry bucket for 5 minutes to allow the glue to dry sufficiently before returning the crab to its compartment in the tank. The whole process lasted no longer than 10 minutes for each crab. Control crabs were also subjected to the handling process of tagging but without placement of a tag to ensure any behavioural differences between the tagged and control group could not be attributed to the handling procedure.

The 2 groups were separated after tags were attached, the tagged group were returned to their compartments in the original tank, the control group were put into compartments in a new tank this was thought appropriate should the glue be toxic to the animals.

Every 24 hours the crabs were checked for mortality and tag retention. To investigate the extent of stress placed on the crab during the tagging process feeding rates of both groups were also observed every 24hrs. Feeding rates were understood to be the wet weight (g) (2.d.p) of fresh mussel meat given to each crab. Any meat remaining after a 24-hour period was reweighed to reveal the weight of meat consumed. The amount consumed was then divided by the total wet weight of the individual crab and changed to a percentage, giving the percentage of body weight eaten.

2.4.1 Capture, mark, recapture study

A field study was conducted to assess the distribution and habitat use of individuals across the shore, and to find a measure of total abundance of the population. The Menai Bridge site (N 53^{o} 2210, W 004^{o} 1643) was chosen for the field experiment due to the high abundance found during timed searches performed earlier in the month. During the first low tide of the day, the Fucoid and Laminaria zone was divided into a rectangle shape using metal stakes as waypoints. This created a grid of six squares on the shore. Due to the shape of the shore each square was not of an equal area. At approximately the centre of each square a circular area was thoroughly searched for *C. pagurus.* All individuals found were tagged as described in section 2.4 and released once the glue was dry to the searched area. The sex, carapace width and area where each crab was found were recorded. All boulders were replaced to reduce disturbance upon the environment. The total number and sex of crabs tagged each day can be seen in table 1. The radius of the search area was increased each day, until day 4, by 1m to observe the level of increase in juvenile *C. pagurus* individuals located when the size of the area was increased (Table 1).

Table 2. Radius of search area and total number of male and female juvenile *C. pagurus* tagged each day from all searched areas of the site at Menai Bridge.

Day Tagged	**Female**	**Male**
1 Radius 1m	32	39
2 Radius 2m	20	45
3 Radius 3m	13	25
4 Radius 3m	10	20
Total	75	129

2.4.2 Population analysis

Data from the mark and recapture study were analysed using the negative method of the Lincoln index as reported in Jackson (1936). The total population was calculated using equation :

$$\frac{\text{Total marked x total caught on recapture}}{\text{Recaptures}}$$

Due to unequal marking each day the number of recaptures per sampling event needed to be corrected to a constant number, following the instructions in Jackson (1936) recaptures from each sampling event were corrected to 100. So 100 animals were tagged during each tagging event and 100 were recaptured during each sampling event. This was calculated using the equation:

$$y_k = \frac{\text{number recaptured x 100 x100}}{\text{number marked x total caught per recapture event}}$$

Where y_k is the corrected number of crabs recaptured for each sampling event. As the number of recaptures per sampling event had been corrected the total number of recaptures was also corrected. This was done by first calculating r, the average ratio of each y value to the value preceding it using the equation:

$$r = \sqrt{\frac{y_2+y_k}{y_1+y_{k-1}}}$$

Using r the corrected total recapture figure known as a was calculated using the equation:

$$a = \frac{y_1+y_{k-1}}{r} - (y_1+ y_{k-2})$$

The corrected values could then be used in the original equation as:

$$\text{Total population} = \frac{100 \text{ x} 100}{a}$$

The error of the population rate was found using the formulae:

$$s^2 = a/r^4[(2-r)^2\, r^2 A_1 r+(1-r)^4(A_2 r^2+A_{k-2} rk\text{-}^2)+(1-2r)^2 A_{k-1} r^{k-1}+A_k r^k]$$

Where s^2 is the standard error of a, and A is the corrected values of recaptures calculated previously.

As juvenile *C. pagurus* were tagged over several tagging events the negative method was used to calculate the death and emigration rate of juvenile *C. pagurus* for the site at Menai Bridge. The death and emigration rate can be described using the equation:

$$\frac{\text{Recaptures}}{\text{number marked}} \times \frac{P_{n+1}}{\text{number caught date}_{n+1}}$$

Where *P* is the total population calculated previously.

Prior to finding the death and emigration rate we need to find the survival rate, to do this the corrected figures calculated previously are used again as the number of individuals tagged at each tagging event was not equal. The survival rate was found using the equation:

$$r\_ = \frac{(y_1)_n}{a_{n+1}}$$

Where *r_* is the survival rate using the negative method.

From the survival rate the death and emigration rate can was found using the equation:

$$D+E = 1- \frac{(y_1)_n}{a_{n+1}}$$

Which is the same as:

$$1- \; r\_$$

To find the birth and emigration rates from the mark and recapture study the number of individuals tagged during each tagging event were corrected in the same way as the recapture data described previously this is the positive method described in Jackson (1936). A positive *r* was calculated (*r*+), this could be used in the equation:

$$1\text{-}r\ +$$

This gave the level of birth and immigration into the population. The death and emigration rates as well as birth and immigration rates could not be separated because the area of each individual square from the total search area was not equal preventing a common ratio of area to be calculated.

2.5 Hepatopancreas dissection

During August a selection of juvenile *C. pagurus* representing different size classes were randomly selected from each sample site. Due to the varying abundance of crabs at each site uneven sample sizes were collected from each site (Table 3). During collection *C.pagurus* were placed into an ice bath and transferred to the laboratory. Upon arrival the crabs were euthanized humanly by piercing the heart through the dorsal shell. They were then placed in the freezer until dissection occurred.

The carapace width to the nearest mm, the weight in grams, to 2 decimal places, and the sex of the individual were all recorded. The carapace was then cut along the cuticle line removing the top of the carapace (Figure4). The hepatopancreas was removed, blotted and the wet weight recorded (g) (2.d.p).

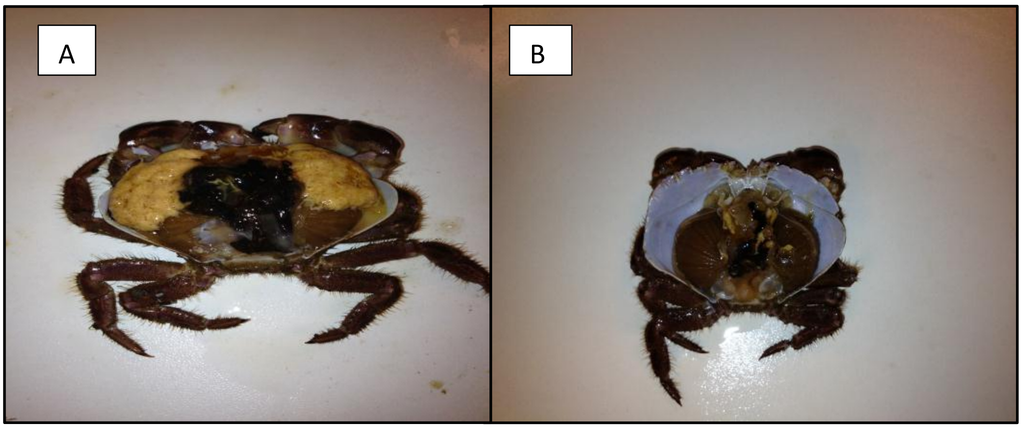


Figure 4 Pictures of juvenile *C. pagurus* with the top of the carapace and cuticle removed, picture A shows the hepatopancreas present and B with the hepatopancreas removed.

2.5 Statistical analysis

2.5.1 Abundance and size analysis

All analyses were performed using SPSS (v.14) and PRIMER (Plymouth Routines in Multivariate Ecological Research, v.6; Clarke & Gorley 2006) statistical packages. Abundance and carapace width data for 2013 was analysed using a 2 level nested general linear model (GLM) with zone nested within site and sampling event. Prior to analysis a Levene's test was conducted to check for homogeneity of variances.

The mean abundance and carapace width data from Menai Bridge and Criccieth for 2012 and 2013 were compared using a one-way ANOVA. Prior to analysis a Levene's test was conducted to test for homogeneity of variances between samples. A two sample Kolmogorov – Smirnov test was performed to analyse the carapace width distribution data from both sites for 2012 and 2013. Abundance and carapace width size data from all sites were collated to give a mean abundance for north Wales. This was compared with mean abundance and carapace width data collected from a study conducted concurrently in south Wales (Hayle, 2013). The mean data were compared using a one-way ANOVA. A Kolmogorov- Smirnov test was used to analyse the carapace width distribution data for north and south Wales for 2013.

2.5.2 Environmental data – biotic factors

A mean species count for each sampling event for each zone of each site was calculated and imported into PRIMER. Diversity measures were calculated using Diverse. The species richness of each site was analysed using univariate testing in SPSS and a one-way ANOVA was used to assess differences in species richness from each site.

Cluster and multidimensional scaling were performed on a Bray-Curtis ranked similarity matrix on fourth root transformed species abundance data. Any large outliers skewing the data were removed from the analysis and the tests rerun allowing relationships to be observed in more detail. Abundance data for *C. pagurus* was overlaid onto the species abundance data using 2d bubble plots to explore levels of juvenile *C. pagurus* abundance with community composition data.

Simper analysis was performed on the biotic data, species contributing to <90% of the cumulative contribution were analysed, indicator species were identified as those which contributed > 1.5 S.D to the similarity contribution. These indicator species were then subjected to univariate testing.

2.5.3 Environmental data analysis- Abiotic factors

A mean of the environmental parameters collected (Appendix B2) for each sampling event was also calculated for each zone at each site and imported into PRIMER. The data was normalised, due to different measurement scales used and a Euclidean distance similarity matrix created. The abiotic data was treated in the same way as the biotic data to examine similarities in the abiotic factors in each sample.

2.5.4 Weight of hepatopancreas analysis

The mean weight of the hepatopancreas for each site was compared using ANOVA, a Levenes test was to test for homogeneity of variance between samples. All data for each site was compiled together and the means of each sex were analysed to identify any differences in size between sexes using a Kruskal – Wallace test. The combined data, including both sexes, was transformed (ln+1) and correlated with the carapace width data to produce a graph examining the relationship between hepatopancreas wet weight and carapace width. A Pearson product – moment correlation was used to look at the significance of this association.

3.0.0 Results

A total of 323 juvenile *C. pagurus* were recorded across 4 sites over 3 sampling periods between 23rd of June and 27th of July 2013 (Table 4). Menai Bridge had the highest total abundance and Criccieth the lowest total abundance. Across all sites 61% of the crabs were observed in the Fucoid zone. The carapace width of the crabs ranged between 10 – 95 mm with a mean width of 35.83 mm ($\pm$ 1.02)

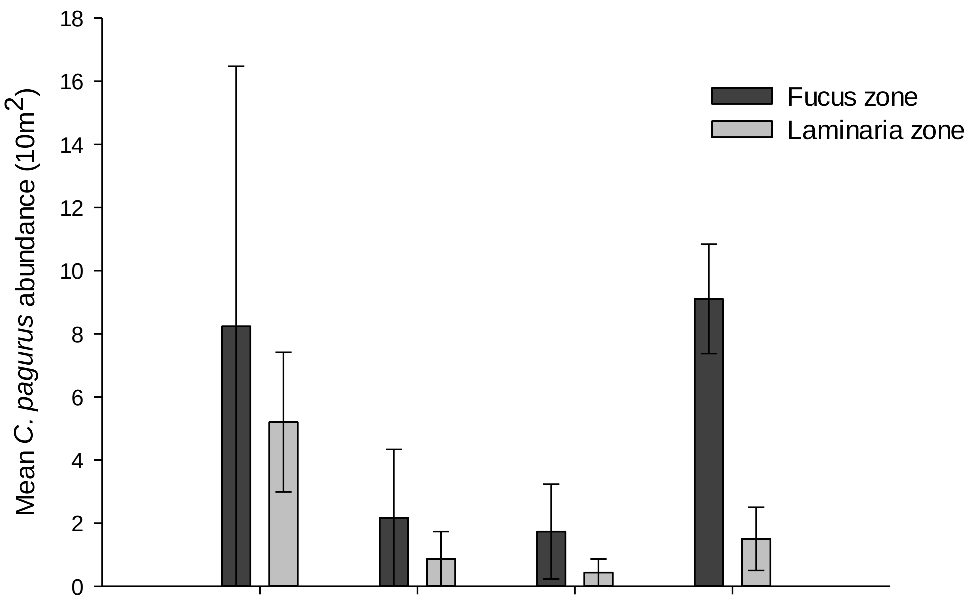
Table 3 total abundance and mean abundance per 10 m^2 for each of the 4 sites surveyed in 2013 in north Wales.

Site Name	**Total Abundance**	**Mean abundance per 10m^2 ($\pm$ S.E)**
Menai Bridge	134	9.68 ($\pm$1.93)
Criccieth	16	1.156 ($\pm$1.09)
Y Swint	79	6.043 ($\pm$0.48)
Llandudno pier	85	6.14 ($\pm$1.62)

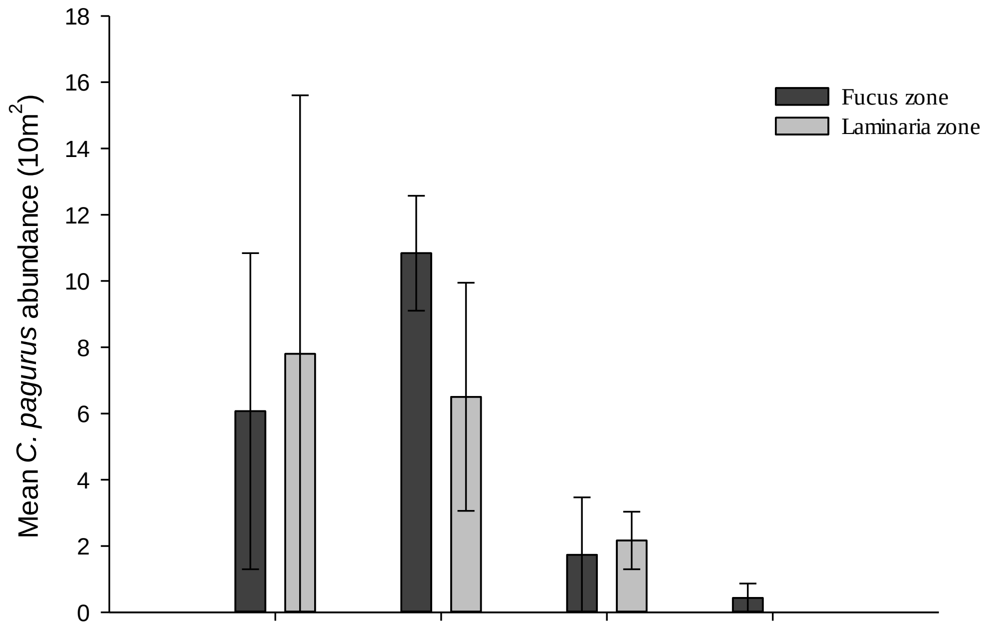
3.1.0 Abundance and size of *C. pagurus* from 2013

No significant difference in the abundance of juvenile *C. pagurus* between sites was found (ANOVA, $F_{1,4}$ = 1.19, P = 0.42) zones (nested within site) (ANOVA, $F_{4,16}$ = 1.33, P = 0.30). There was a significant difference between sampling events (ANOVA, $F_{3,4}$ =16.79, P=0.02)(Figure 5)

1)



2)



3)

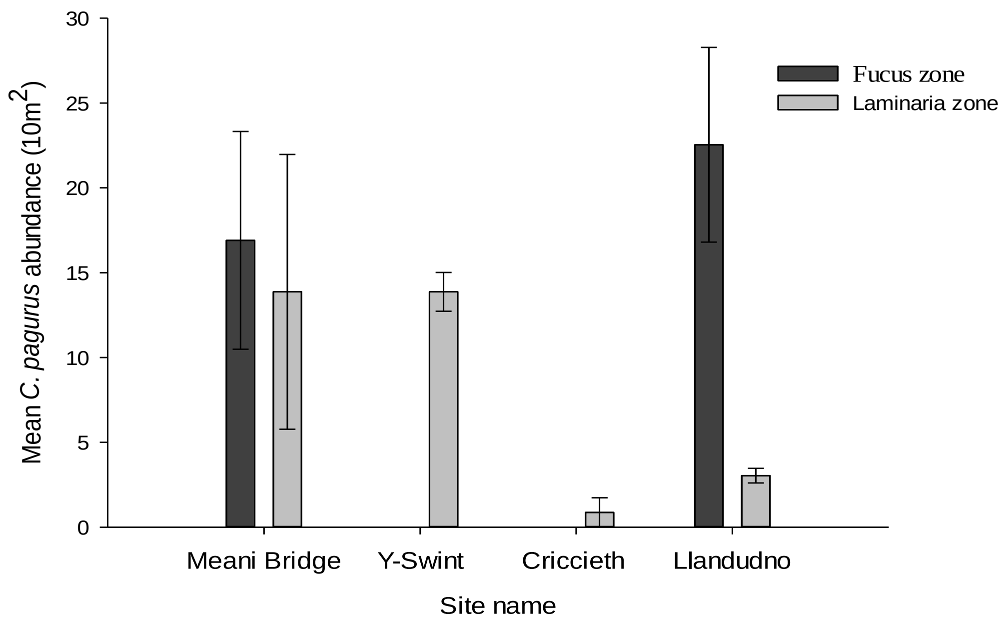


Figure 5 Mean abundance of juvenile *C. pagurus* per $10m^2$ ($\pm$ S.E) recorded in the *Laminaria and Fucoid* zone of the shore for each sampling event 1) first sampling event 2) second sampling event 3) third sampling event.

No significant difference was found in the carapace width between sites (ANOVA, $F_{3,4}$ =2.356, P0.788), zones (nested within site) (ANOVA, $F_{4,16}$ =2.237, P = 0.065) and sampling event (ANOVA, $F_{1,4}$ =31.845, P =<0.001) (Figure 6).

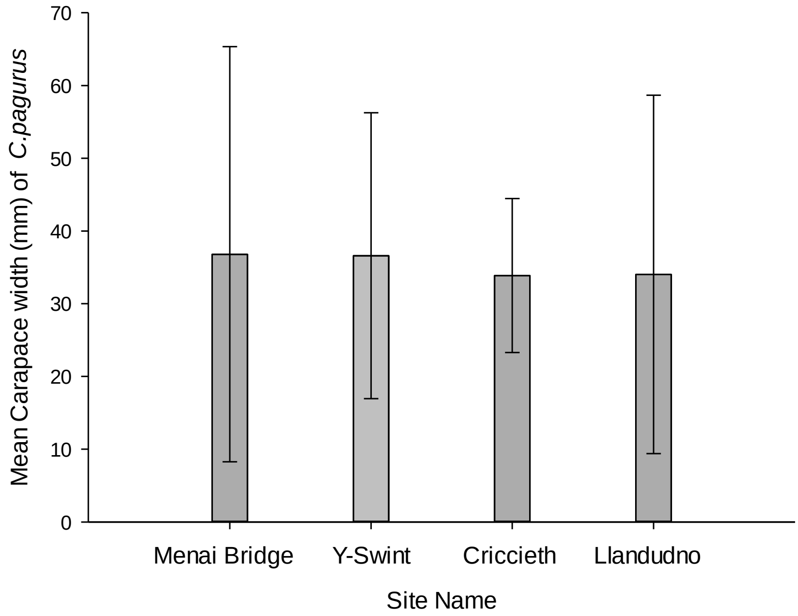


Figure 6 Mean carapace width (mm) of juvenile *C. pagurus* recorded from *Laminaria* and *Fucoid* zone from 3 sampling events.

3.1.1. Comparison of abundance and size of juvenile *C. pagurus* from 2012 and 2013

There was a significant difference in the mean abundance of juvenile *C. pagurus* found at Menai bridge between 2012 and 2013 (ANOVA, $F_{1,32}$ = 21.204, P <0.01). A significant difference was also found between the mean carapace width in 2012 and 2013, (ANOVA, $F_{1,393}$ =8.811, P <0.01). A significant difference was also found between the size distribution between the two years with 2013 having a higher percentage proportion of smaller individuals and 2012 having a higher percentage proportion of larger individuals, figure 7, (Kolmogorov-Smirnov, Z= 1.736, P = 0.05).

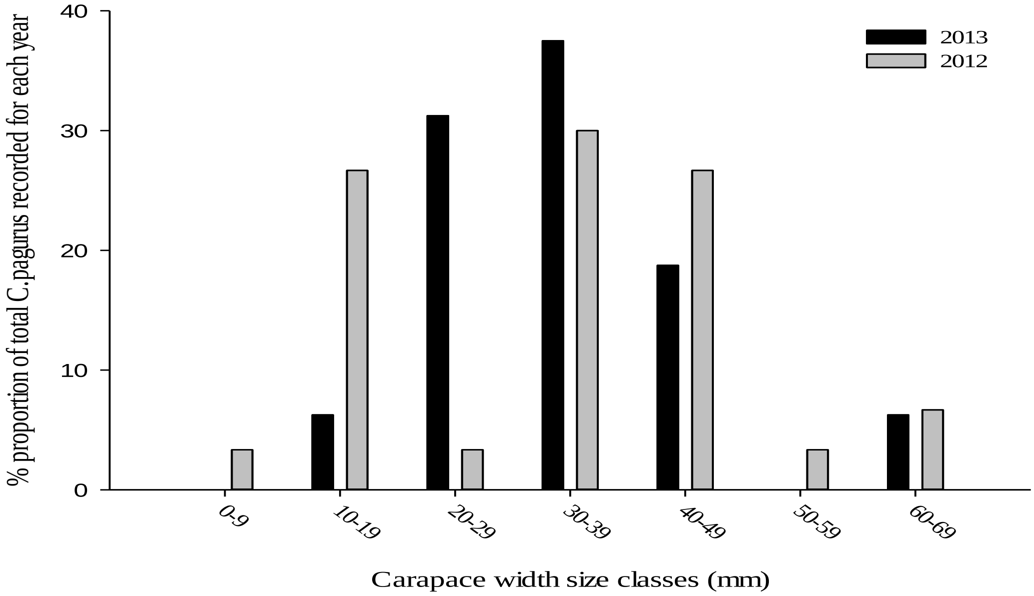


Figure 7 The distribution of percentage proportion of carapace width size classes (mm) of juvenile *C.pagurus* recorded at Menai Bridge in the *Laminaria* and *Fucoid* zone across 3 sampling events for 2012 and 2013.

There was no significant difference in the mean abundance of juvenile *C. pagurus* at the Criccieth site between 2012 and 2013 (ANOVA, $F_{1,16} = 3.628$, $P = 0.075$). No significant difference was found between the mean carapace width of juvenile *C. pagurus* in Criccieth from 2012 and 2013 (ANOVA, $F_{1,44} = 0.008$, $P = 0.931$). There was also no significant difference in the distribution of size classes between 2012 and 2013 (figure 8) (Kolmogorov-Smirnov, $Z = 0.767$, $P = 0.598$).

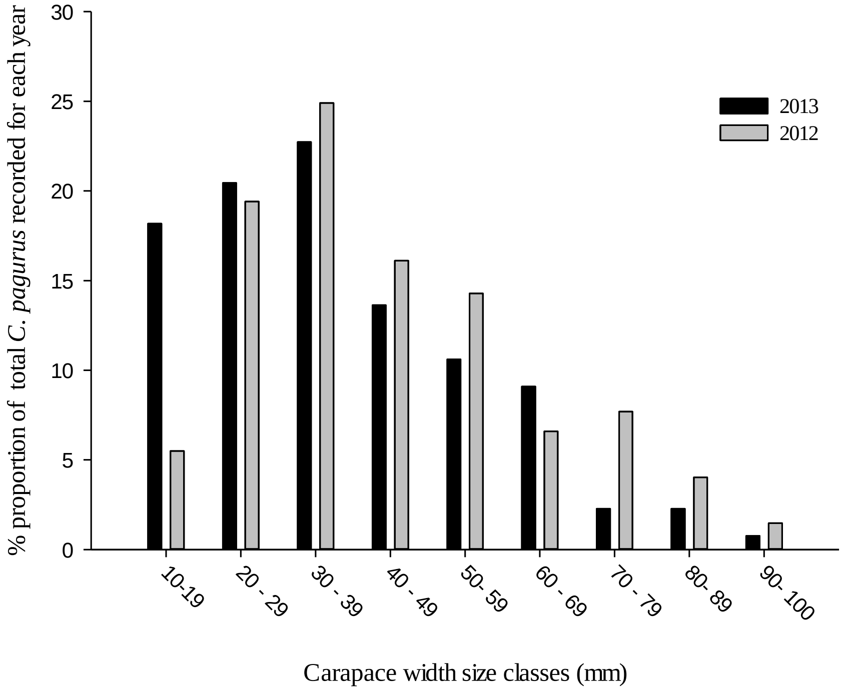


Figure 8 The distribution of percentage proportion of carapace width size classes (mm) of juvenile *C.pagurus* recorded at Criccieth in the Laminaria and Fucoid zone across 3 sampling attempts for 2012 and 2013.

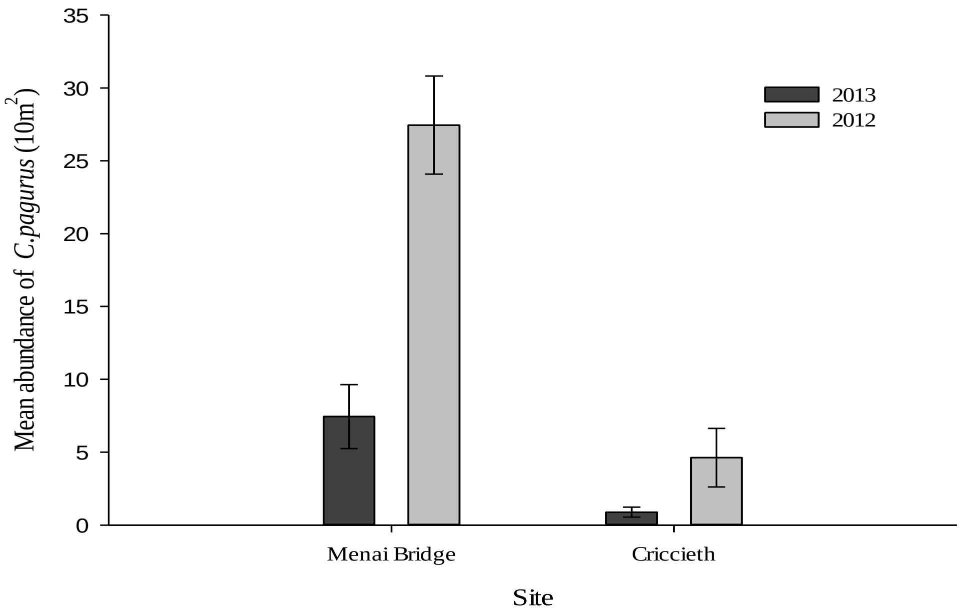


Figure 9 Mean abundance of juvenile *C. pagurus* per 10m^2 ($\pm SE$) across both *Laminaria* and *Fucoid* zone from Menai Bridge and Criccieth sites from surveys completed in 2012 and 2013.

In north Wales a higher percentage proportion of individuals had a smaller carapace width and the proportion declined in the large size classes. The percentage proportion in south Wales displayed a more uniform spread and a wider range of size classes extending beyond those found in north Wales (figure 9). The distribution of data was found to be significantly different (Kolmogorov - Smirnov, Z = 3.582, P <0.01). The mean carapace width in north Wales was 37 mm compared to 46mm in south Wales. This was found to be significantly different (ANOVA, F_1 =43.401, P <0.01).

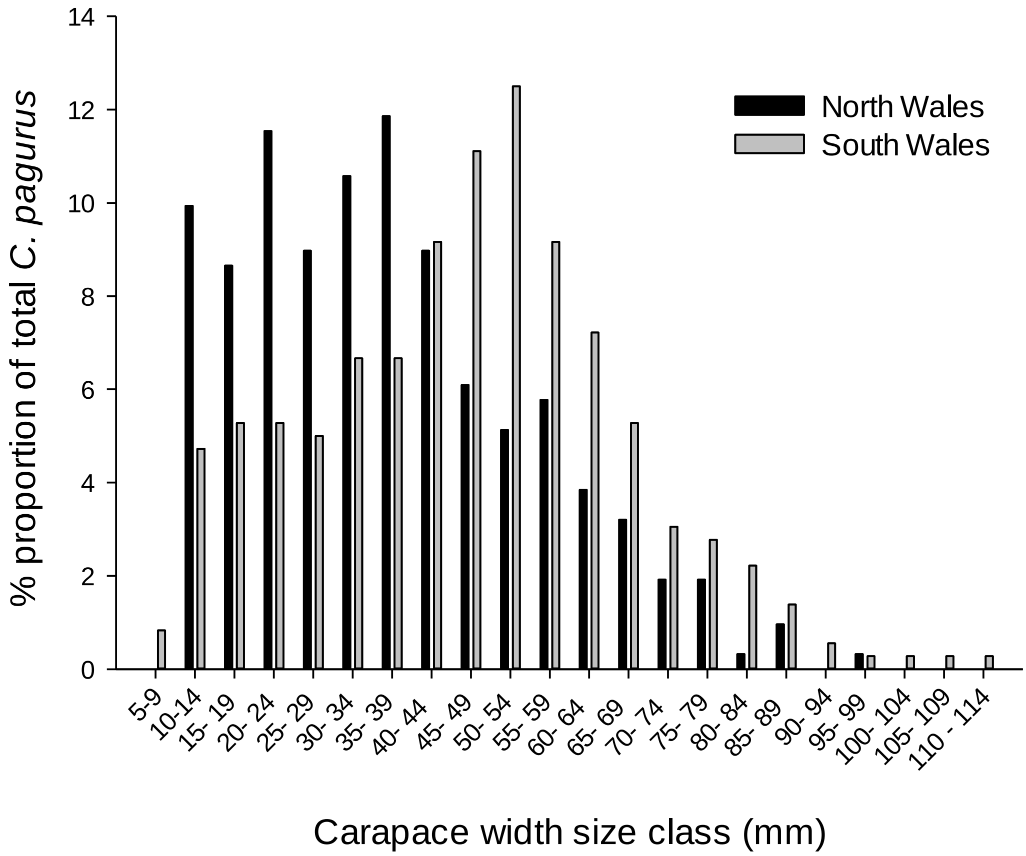


Figure 10, Percentage proportion of juvenile *C. pagurus* recorded in each size class from 5 sites in south Wales and 4 sites in north Wales across *Laminaria* and *Fucoid* zone during 2013

3.2. Environmental data – Biotic factors

No significant difference in species richness was found between site (ANOVA, $F_3 = 0.277$, $P = 0.840$) (Figure 11) zones (nested within site) (ANOVA, $F_4 = 2.587$, $P= 0.077$). There was a significant difference in the abundance levels between sampling events (ANOVA, $F_4 =2.587$, $P = 0.077$)

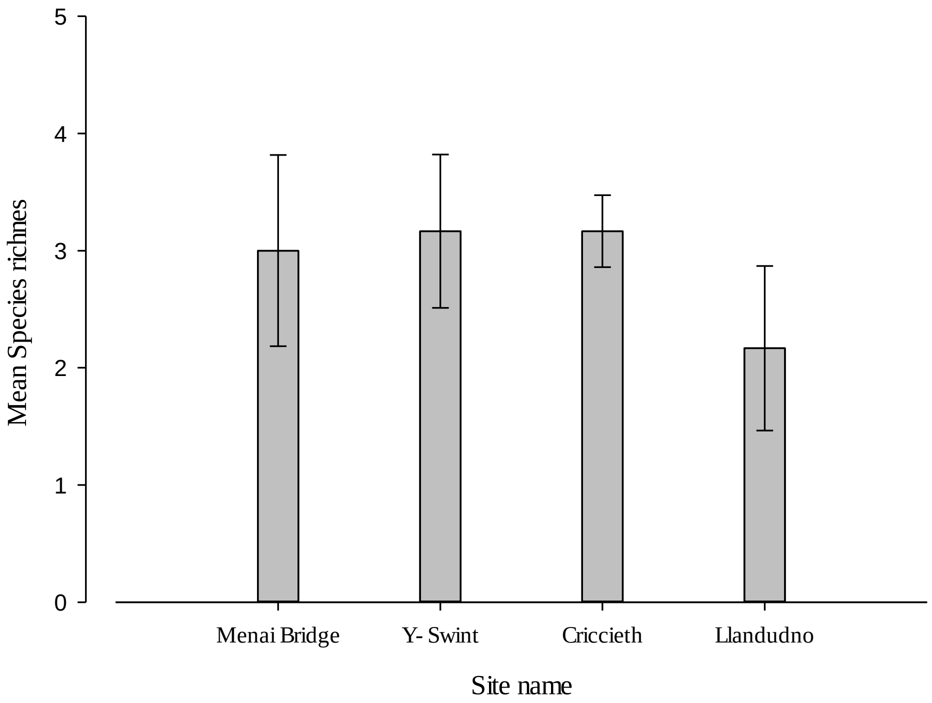


Figure 11 Mean species richness for each of the 4 sites surveyed over 3 sampling periods

The MDs plot, Figure 12 indicated there was an association in species composition between the 4 sites or zones surveyed. This was found to be significant (ANOSIM, $R = 0.293$, $P = 0.01$).

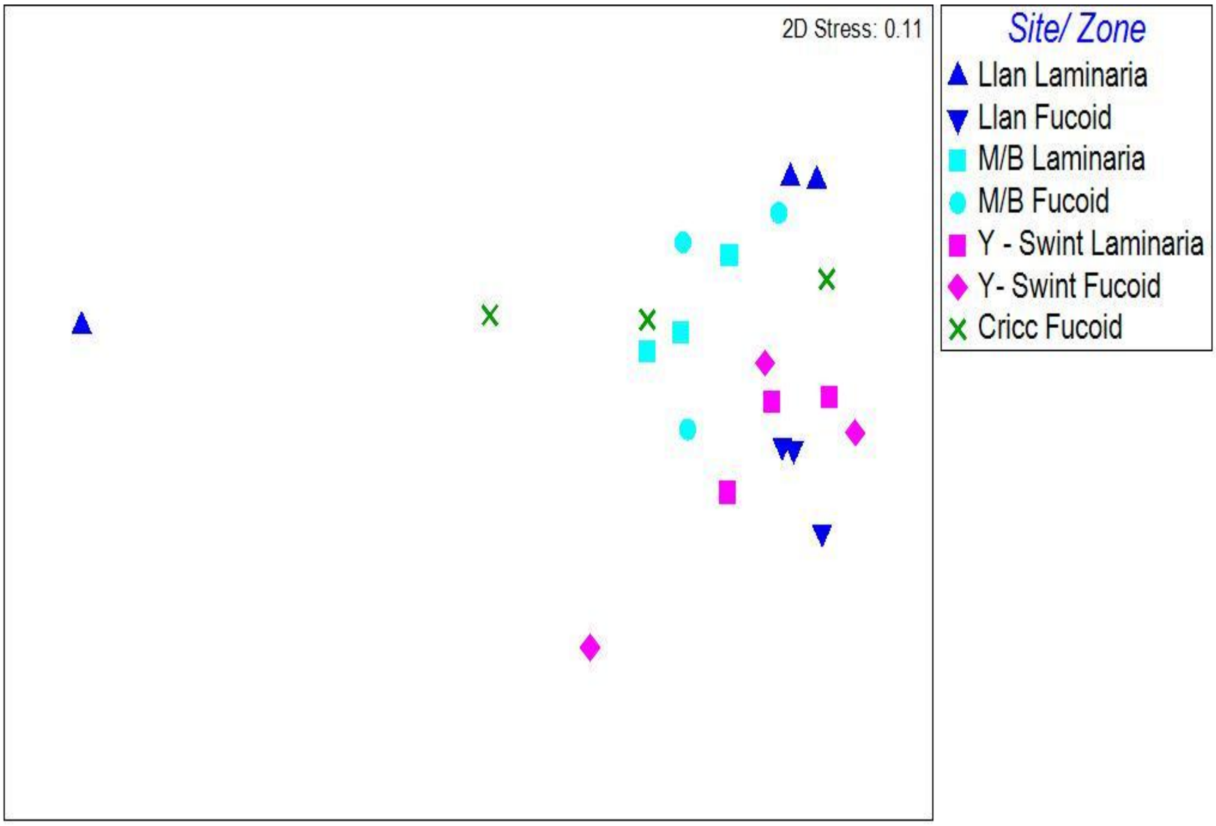


Figure 12 MDS plot of the mean species abundance for each sampling period for each zone of each of the 4 sites surveyed.

An overlay of *C. pagurus* abundance data onto the species composition data indicated that there area with a higher abundance of juvenile *C. pagurus* were found in areas with similar species composition, Figure 13.

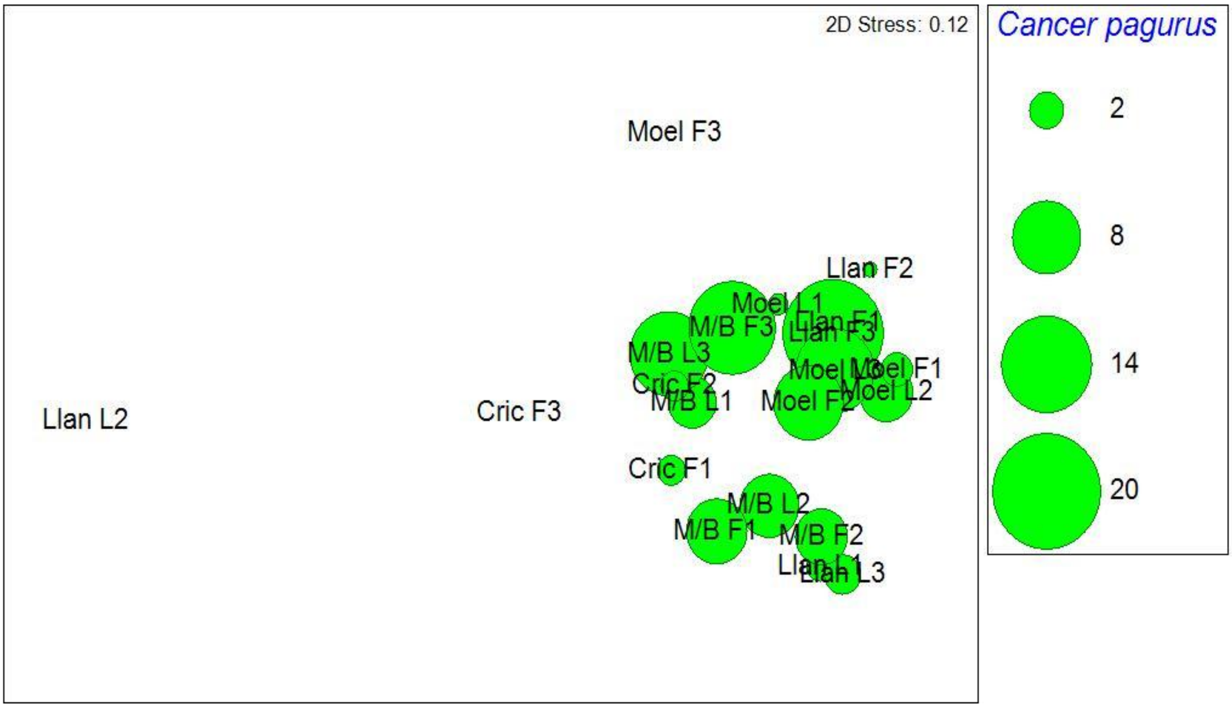


Figure 13 2d bubble plot of mean abundance data for each sampling event for juvenile *C. pagurus* for each zone of each of the 4 sites surveyed.

From the simper analysis 3 indicator species were highlighted from the species composition data. All the indicator species were found to be non-significant across site, zone nested within site and time except *Littorina saxatilis* which was significant between zone nested within site (Table 4).

Table 4 Nested ANOVA outputs for indicator species (obtained from simper analysis) for site, Zone (nested within Site) and time. * indicates significant results.

Species/Site/Zone/Time	**d.f**	**F**	**P**
Littorina Obtusata			
Site	3	2.219	0.131
Zone (Nested within Site)	3	0.035	0.991
Time	3	1.127	0.396
Littorina saxatilis			
Site	3	0.280	0.838
Zone (Nested within site)	3	4.906	0.016*
Time	3	0.216	2.16
Mytilus edulis			
Site	3	1.887	0.307
Zone (Nested within site)	3	2.132	0.142
Time	3	4.945	0.113

3.2.1 Environmental data – Abiotic factors

The abiotic factors expressed some clustering with Menai Bridge Laminaria zone and

Llandudno Fucoid zone (Figure 14), this was found to be non-significant (ANOSIM, R = -0.044, P = 0.737).

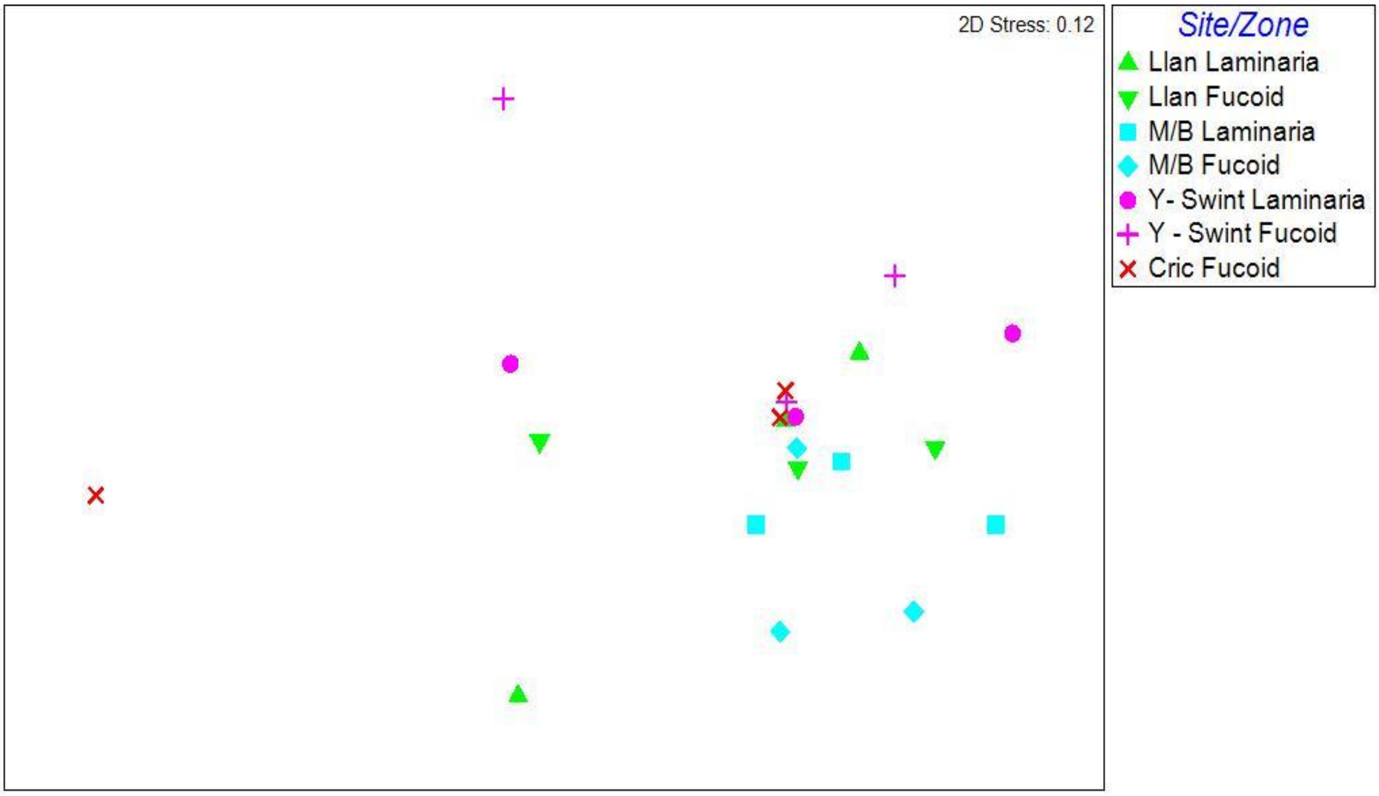


Figure 14 MDS plot of mean biotic environmental data for each site and zone over 3 sampling periods.

From the simper analysis 6 abiotic parameters were highlighted for further investigation into their presence across sites and zones (table 5).

Table 5 Nested GLM results, for abiotic parameters highlighted from the simper analysis, across site, zone (nested within site) and sampling event (time).

Species/Site/Zone/Time	d/f	F	P
Boulders			
Site	5	4.787	0.009*
Zone (nested within Site)	5	0.299	0.608
Time	1	32.129	0.122
Fucus serratus			
Site	5	1.421	0.276
Zone (nested within site)	5	4.540	0.86
Time	1	1.877	0.402
Sand			
Site	5	3.015	0.047*
Zone (nested within site)	5	0.114	0.750
Time	1	76.189	0.100
Ulva lactuca			
Site	5	0.756	0.596
Zone (nested within site)	5	0.454	0.530
Time	1	11.133	0.194
Barnacle sp			
Site	5	1.086	0.410
Zone (nested within site)	5	1.961	0.220
Time	1	3.032	0.334

3.3 Mark and recapture tag retention study

All tags successfully remained on the carapace throughout the tag retention study, no behavioural differences were observed between the tagged and control group throughout the study, with one of the larger tagged individuals proceeding to moult. Survival of the individuals was lower in the tagged group with a survival percentage of 75% compared to

87.5% for the control group. Mortality occurred two days after the tagging procedure for all animals.

Feeding rates were significantly higher in the tagged group compared to the control group ($F_1 = 7.285$, $P = 0.019$). The tagged group show a higher variance in feeding rates between individuals. The control group show a reduction in feeding rates on day three after they were handled. The tagged group show the same drop in feeding rate but a larger reduction compared to the control group. The individual that moulted through the study ceased feeding from day 4 onwards (Figure 15).

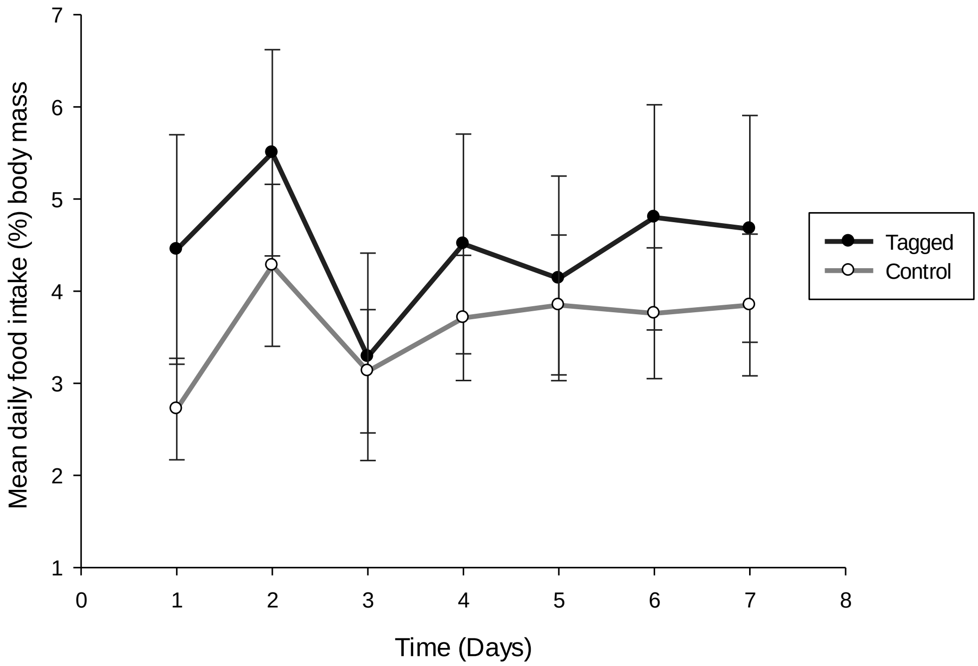


Figure 15 Mean daily % $\pm$ S.E. of body mass intake of fresh mussel meat by crabs over a 7-day period; tagging occurred on day 3, under laboratory conditions.

3.3.2 Capture, mark and recapture study

Throughout the study uneven numbers of individuals were caught on each of the tagging events across the increasing search area, (table 6).

Table 6 The total number and mean number of crabs caught in each search area that was extended by 1m each day.

Length of search rope (m)	Total caught in day	Mean caught in day
1	77	12.83
2	66	11
3	72	14.4

The 1m search area had the highest catch with the 2 m search area having the lowest total catch, the 3m search area did have the highest mean catch in that area. There was no significant difference between these size differences ($F_2 = 0.141$, $P = 0.870$).

A total of 216 crabs (77 females and 136 males) were tagged over 4 consecutive tagging days (table 2 section 2.4.1), 19 were recaptured over 5 search days (15 males and 4 females) (Table 7). 4 individuals were caught on 2 occasions. The carapace width ranged between 9 mm and 89 mm.

Table 7, Number and sex of crabs recaptured at each recapturing event throughout the study.

Recapture Day	Female	Male
1	1	0
2	2	3
3	0	4
4	1	8
Total	4	15

All recaptured crabs were found in the same tagging area they were initially marked and exhibited minimal movement.

Males had a recapture rate of 7% compared to females with a recapture rate of only 2%, this was found to be non-significant and both sexes were considered together with a total recapture rate of 9%. There was no significant difference in the rate of recapture between sexes compared to the number tagged (χ^2 = 2.060, 1df, P = 0.151). The total recapture rate is similar to that of other studies (Silva *et al* 2013).

The total population estimate calculated = 9442 (± 146.65 individuals). Death and emigration rates = -1.11 with birth and immigration rates = 0.955.

It should be noted that during the procedure of administering tags in the field some individuals were damaged due to antagonistic behaviour when placed in the dry buckets.

3.4 Hepatopancreas analysis

There was no significant difference in the mean weight of hepatopancreas between 4 sites surveyed during 2013 (ANOVA, $F_3 = 0.702$, $P = 0.555$) (Figure).

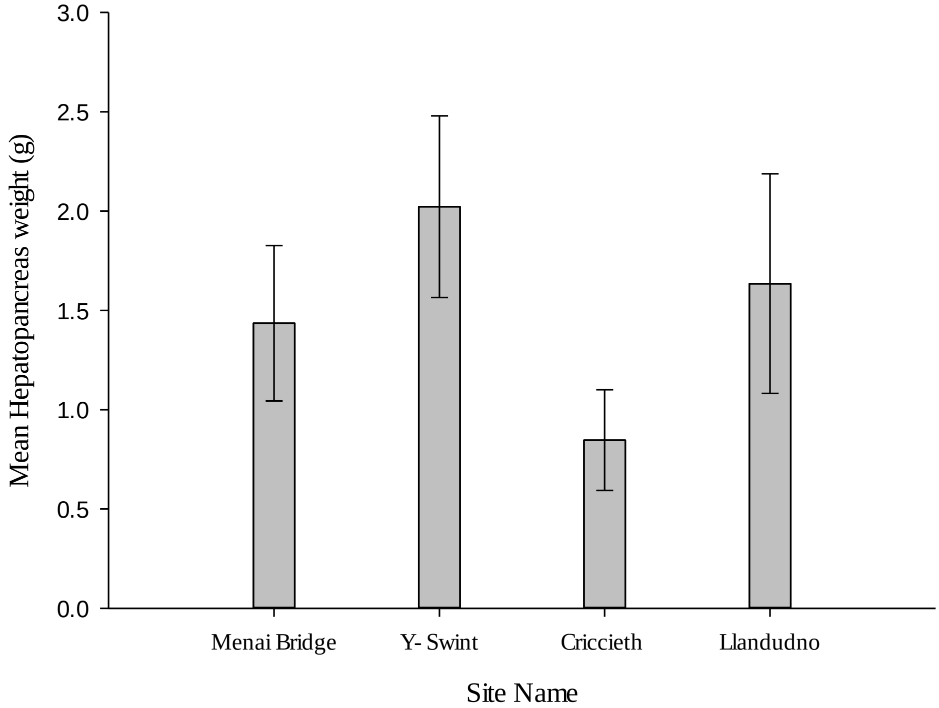


Figure 16 The mean hepatopancreas wet weight (g) of individuals from each site

As there was no significant difference between the sites the data was collated and the means of the sexes were analysed. A significant difference was found between the mean weight of the hepatopancreas, with females having a higher mean wet weight (Kruskal Wallace, $\chi^2 = 13.999$, $P = 0.000183$).

A significant positive trend was found between the carapace width of the all the individual crabs recorded and the ln hepatopancreas wet weight (Pearson Correlation, r =0.793, P <0.01) , (Figure 17).

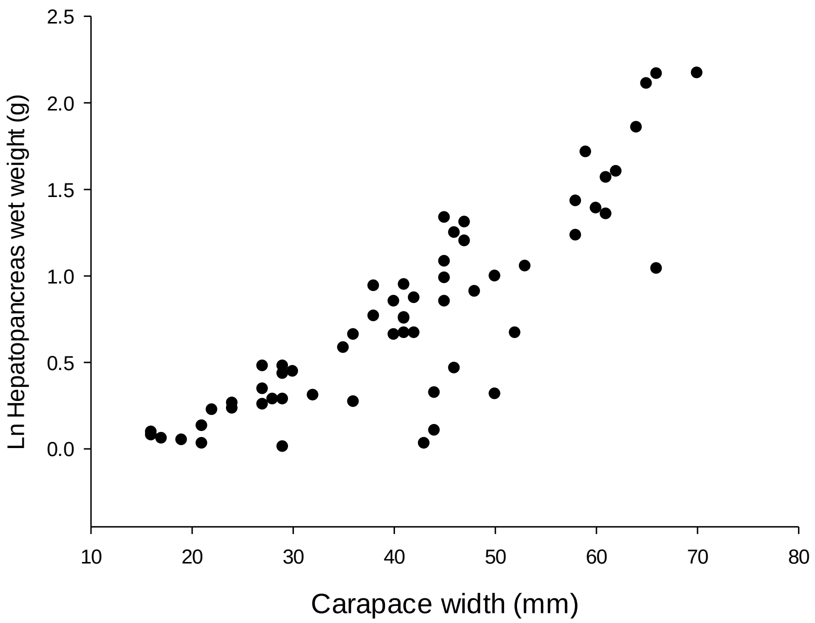


Figure 17 A correlation of individual juvenile *C. pagurus* carapace widths (mm) with the ln wet weight of the hepatopancreas (g) from all 4 sites surveyed in one sampling period in 2013

4.0 Discussion

This study has successfully achieved the objectives stated in the introduction, with comparisons made with the previous year's data to describe any inter annual differences in the abundance, size and distribution of juvenile *C. pagurus* between 2012 and 2013. It was stated in Dickinson 2012 that the presence of juvenile *C. pagurus* on the shore is limited as only 28% of the original shores recorded individuals, this study found similar behaviour with only 25% of the original sites visited having a presence of juvenile *C. pagurus*. Results from the present study have found no significant difference in the abundance of juvenile *C. pagurus* between the sites and the zone surveyed however there was a difference in abundance over time. Changes in abundance may be due to larvae settling into the intertidal zone continuously throughout the spawning season, *C. pagurus* are known to release young throughout the summer in pulses as first stage zoeal larvae have been caught in plankton samples from July to October in the North Sea (Nicholas *et al* 1982). Abundance may also change to due mortality and emigration from the area, as small juveniles, *C pagurus* are extremely vulnerable to predation and changes in the environment (Gosselin 1997). As they grow a subtle ontogenetic shift in habitat and diet has been reported by Gosselin (1997) suggesting some of the juveniles may have dispersed from the immediate survey area although abundances between zones did not change during this study so any trends in distributions was not detected during sampling.

There was no significant difference in carapace width across sites and between zones, this might be due to limitations of the methodology preventing trends in size class separation being highlighted. Dickinson (2012) explained spatial segregation used in the methodology might not have been large enough to detect changes in trends between algal zones. Further sampling sub tidally may be necessary to increase the geographic area surveyed.

Reconnaissance dives suggested by Dickinson (2012) would allow area rarely revealed by the tide to be included in the study. Other sampling methods could be incorporated, for example baited pots which have been used successfully by Silva *et al* (2013) to observe the abundance of a variety of crab species including *C. pagurus* throughout the tidal cycle of intertidal areas.

Other studies have also found trends in size class distribution difficult to observe. A mark and recapture study conducted by Silva *et al* (2013) found *C. pagurus* use the entire intertidal area regardless of size. Of those individuals marked on the lower shore, 35.4% were recaptured on the upper shore. Refuge from large predators such as fish (Wahle 2003 & Silva *et al* 2013) whilst foraging is thought to cause a migration up shore. That study, which was conducted over a period of a year, did not collect enough recapture data to fully understand shore height fidelity, and ontogenetic shift in habitat use due to size may still occur (Silva *et al* 2013).

Size class data from this study were significantly different to the size class distribution of 2012 with crabs being of a smaller size during 2013. There were a larger percentage of crabs found in the *Fucoid* zone in 2013 compared to a higher percentage found in the *Laminaria* zone in 2012. This does indicate that crabs of a smaller carapace width might live higher on the shore compared to those of a larger carapace width found in the Laminaria zone but further studies would need to be conducted to conclude this hypothesis.

An alternative theory to the presence of more crabs in the Fucoid zone during 2013 compared to 2012 may be that the crabs may have settled into the middle of the habitat, due to settlement potentially occurring later in the year of 2013 they have not been physically able to move to the edges of the habitat due to their smaller size. This is supported by the mark and recapture study which tagged a larger proportion of crabs in the middle Fucoid area compared to the search areas on the edge of the habitat. This, of course could be simply because the environment is more suitable for juvenile *C. pagurus* in the middle Fucoid area than towards edge.

4.1.0 Differences in abundance between 2012 and 2013

Two sites, Menai Bridge and Criccieth, were sampled during June and July of 2012 and 2013. This allowed a comparison of the abundance and carapace width of the individuals recorded to investigate any interannual differences. The results showed that there was a difference in the mean abundance and carapace width at Menai Bridge but not Criccieth, with Menai Bridge having a higher abundance and larger mean carapace width during 2012. The distribution of the size classes was also found to be different with more individuals being recorded in the larger size classes during 2012 compared to 2013. Differences in abundance level could be a result of experiment bias, as surveyors have not been consistent through both studies. The surveying method requires that the sampler lift and move large boulders to reveal hidden crabs. Results may be skewed due to differences in the sampler's ability to lift and move such boulders.

Physical and biological factors such as food availability, salinity and temperature may explain inter annual differences in abundance and carapace width between the 2 years. Temperature is usually thought to be a dominant factor (Weiss *et al* 2009). Average temperatures in the spring were higher in 2012 (10.5^{o}c) compared to 2013 (8.6^{o}c). *C. pagurus* exhibit a linear relationship between temperatures and instar development (Weiss *et al* 2009), as *C. pagurus* are completing the planktonic larval stage during spring they may have settled into the intertidal zone earlier, although at a smaller body size in 2012. This is because the higher water temperature reduces the intermoult period for crustaceans in turn reducing their larval duration (Robinson & Tully 2000). There is a threshold where temperature becomes too high causing larval mortality due to the high-energy costs placed on the individual at those higher temperatures (Weiss *et al* 2009). Lower temperatures reduce feeding rates preventing the individual from receiving sufficient energy needed for growth (Weiss *et al* 2009). Temperatures below 7 ^{o}c prevent survival past the first zoeal stage (Nicholas et al 1982). During the juvenile stage the higher temperatures reducing intermoult periods allows the individual to obtain a larger size over a shortened period of time explaining larger carapace widths onshore during 2012 (Fisher 1999). A similar trend can be seen in the abundance and size of juvenile *C. pagurus* in south Wales compared to those recorded in north Wales during 2013. Sea surface temperatures could not be obtained for this area to prove this.

As physical and biological factors may have influenced the settlement potential of the juvenile crabs before they arrived at the sites it further highlights the importance of information needed for the entire life cycle of *C. pagurus* in north Wales. Information also needs to be collected on the pre settlement processes that are imposed on the larvae and how these may affect settlement into the benthic habitat. Ontogenetic shifts between megalopae and juveniles have been observed in other crustaceans studied in Chile, megalopae settled into one microhabitat and upon metamorphosis into a juvenile migrated to an alternative microhabitat. This shift can occur over a relatively short period of time and may not be observed unless intense sampling is performed (Pardo *et al* 2007). Knowledge of settlement cues for megalopae will be beneficial to understand why juveniles occur in one habitat and not another despite appearing similar. This will allow fisheries managers to fully understand the recruitment process into the adult population and harvest sustainable numbers of *C. pagurus*. Field experiments which have been manipulated with artificial microhabitats can be used to investigate which microhabitats encourage the highest settlement of *C. pagurus* megalopae (Pardo *et al* 2010).

4.2. Environmental parameters

All the environmental parameters recorded showed no significant difference between sites, zones and sample events with the exception of boulders which were significantly different between sites. The presence of boulders creates a structurally complex environment for the crabs providing them with refuge from predators and physical conditions they may be vulnerable to, promoting their survival (Robinson & Tully 2000). Many crustacean species have been recorded settling into structurally complex habitat such as the Blue crab, *Callinectes sapidus,* which will settle into seagrass beds upon first settlement before dispersing to other habitats as their size increases (Etherington & Eggleston 2000). The results from this study do not reveal any environmental parameters which may be important cues for *C. pagurus* settlement. This is different to results from the previous year's study Dickinson (2012) which revealed positive correlations between abundances of *C. pagurus* and prey species such as *Porcellana platycheles*, *Littorina littorea* and *Nucella lapillus* (Neal & Wilson 2008). Also abiotic factors such as sediment were positively correlated with abundances of *C. pagurus*. Trends in environmental data important to juvenile *C. pagurus* may not have been detected in this study because of a reduction in sites surveyed in 2013 compared to 2012, this was due to a lack of new sites found with juvenile *C. pagurus* present.

4.2 Mark and recapture preliminary study

During the tag retention study, feeding rates dropped in both the tagged and control groups on the day when tagging occurred. Handling is stressful for the animal causing it to exhibit predator avoidance behaviour. To maintain this behaviour crustacean's release crustacean hyperglycaemic hormone (CHH), this elevates haemolymph glucose concentrations. After the stressful period the crab needs to restore its bodies to homeostasis and restore energy reserves. Lobsters have been recorded remaining motionless with an increase in oxygen consumption after a handling event for approximately 24hr whilst homeostasis takes place (Jensen *et al* 2013). This may explain the observed decrease in feeding rates in the crabs directly after tagging. Depletion in energy reserves and perhaps hunger may have caused an increase in feeding rates the day preceding the tagging event.

Results from the feeding study concluded the tagged group had a significantly higher feeding rate throughout the whole tag retention study. One individual in the tagged group did eat a large percentage of mussel meat compared to it body size represented by the large error bars (figure15). Post hoc research has found that the individual may have been parasitized, recognised by lack of the usual pigmentation of *C. pagurus* (figure 13). This individual would have increased its food intake due to parasite induced physiological starvation (Stentiford *et al* 2002). During this study this individual suffered mortality, this may have been induced by the stress endured due to the tagging process however, the impacts of stress may have been exacerbated by the parasite. Stentiford *et al* (2002) found survivability was reduced in *C. pagurus* parasitized by *Hematodinium* sp during handling and transporting as the crabs have a reduced carbohydrate and amino acid concentration in their tissues preventing them from recovering effectively from stressful events.

Tank conditions for the control and tagged groups may not have been the same. Temperature, salinity and dissolved oxygen were not actively measured and controlled but were maintained within normal tolerable levels for *C. pagurus*. This may have resulted in variances between the two tanks. Temperature is known to affect feeding rates in crustaceans of all size classes with higher temperatures generally increasing the rate of feeding (Wyban *et al* 1995).

Although feeding rates have been used before to quantify the effects of stress placed on the individual during tagging such as Sharp *et al* (2000) other measurements such as oxygen consumption, nitrogen excretion and levels of CHH present in the haemolymph may be more accurate measurements of stress although would require a more invasive method (Jensen et al 1975, Palterson & Dick 2007).

The effect of the tag on the growth of the individuals was not investigated. The weight of the tag was negligible so it was assumed that it would not cause the crab any extra energy requirements. The long-term effects of the glue on the survival and growth were not measured during the preliminary study due to time constraints. The glue may have been absorbed by the crab through its carapace which has many high density flexible canals which travel through the exoskeleton to the cuticle allowing the transport of ions and nutrients when the animal moults (Chen *et al* 2008). The glue may have penetrated through the exoskeleton this way.

This study identified a form of fast drying glue which allowed tagging to take place in the field reducing stress to the animal when transporting them to the laboratory. It should be noted however that the glue is very powerful and should only be used once the handling procedure manual is read. A mark and recapture study was attempted in Dickinson (2012) but was unsuccessful in recapturing any individuals. This study increased the tagging effort leading to an increase in recaptures which could be reliably analysed. As there was no significant increase in the number of individuals caught with the increasing search area it appears that number of tagging events improves tagging effort. Despite the improvements of the method from 2012, there are still areas of this study that could be improved to investigate the movements and habitat use of the crabs at Menai Bridge. Increasing recapture events would provide a more accurate survival rate estimate. Recapture events for this study occurred on the morning low tide after 2 high tide events, additional sampling during the evening low tide might indicate a more detailed movement pattern. Crabs are thought to forage during the night (Lawton & Hughes 1985) and by sampling on the early and late low tide it might be found that the crabs are limited in their movement during the day and move over a larger area at night.

Recaptured exhibited high site fidelity, this may have been a result of their moult cycle as crabs that are about to enter ecydis stay in the same position (Edwards 1966). The height of the tide may also have been an influence. Tagging and recapture events took place during the neap tides (< 2m C.D). The amplitude of the tide does not change greatly so the crabs may be limited in their movements. This study should have been conducted over a longer period of time to detect differences between tidal cycles. Due to the tide remaining high throughout the study one area could not be accessed and tagged crabs may have been missed during recapture events.

Tag loss was thought to be negligible during the field study as no tags were lost during preliminary testing in the laboratory, however laboratory conditions were not completely representative of field conditions. *C. pagurus* bury into soft substrate under structures that offer them shelter (Lohrer *et al* 2000, Bobelmann *et al* 2007). The crabs in laboratory conditions did not exhibit this behaviour, as no sheltering structures were available to them. Crabs in the field, upon burying themselves, may remove the tag through abrasion. To check the reliability of tags a method of double marking can be used, where two different types of marks are used to test the reliability of both (Henry & Jarne 2007).

The survival rate calculated was of the entire population and did not provide survival rates at different carapace widths. As mortality is usually higher in smaller individuals due to their vulnerability to predation and environmental parameters (Moksnes 2004) a measurement of mortality for each size class would be beneficial for a better understanding of the life cycle.

During marking in the field some crabs were damaged when placed into the dry bucket with conspecifics, damage mainly included autonomy of the legs with one individual losing their claw. Many crab species are cannibalistic and *C. pagurus* are noted to be antagonistic in laboratory experiments (Amaral *et al* 2009). Autonomy is a defence mechanism used by crustaceans to aid predator avoidance and is beneficial for short-term survival. In the long term the loss of a limb can have a detrimental effect on the individual, the extent of this effect depends on which limb is lost, if a claw is damaged or lost the individual suffers a reduction in feeding rates as handling time is increased reducing growth. The loss of a leg appears less detrimental, however growth is again reduced as energy needs to be utilised to replace the lost limb causing a smaller growth increment (Juanes and Smith 1995). Davis (1981) reported an extra 33 weeks was required for spiny lobster *Panulurus argus* to reach legal landing size after autonomy. As *C. pagurus* is a harvestable species this should be taken into consideration if a similar mark and recapture study is to take place again.

4.3 Hepatopancreas

Studies conducted on the hepatopancreas revealed that there was no significant difference in the wet weight between individuals from different shores. This suggests that all sites where juvenile *C. pagurus* are present provide similar nutrition. The number of crabs collected from each site for this study was low to reduce the impact on the population when removing individuals. However sample sizes may have been too small preventing a true representation of the population. Anomalies were found in the data with some individuals having smaller than expected hepatopancreas to carapace widths, the moult stage of the individual might explain this. This is because glycogen, which is stored in the hepatopancreas pre moult, is used up during ecydis and is mostly depleted post moult leading to a smaller sized hepatopancreas (Lockwood 1968).

Disease and parasitology is one aspect possibly affecting growth of individuals and is not widely discussed, due to lack of knowledge (Stentiford 2008). Many of the crabs appearing to be anomalies were collected from Menai Bridge, 2 of these crabs had no pigmentation. *post hoc* research has revealed that these crabs may have been parasitised by *Fecampia erythrocephala* Figure 18. Parasitism may reduce the crab's ability to store extra nutrients reducing the size of the hepatopancreas.

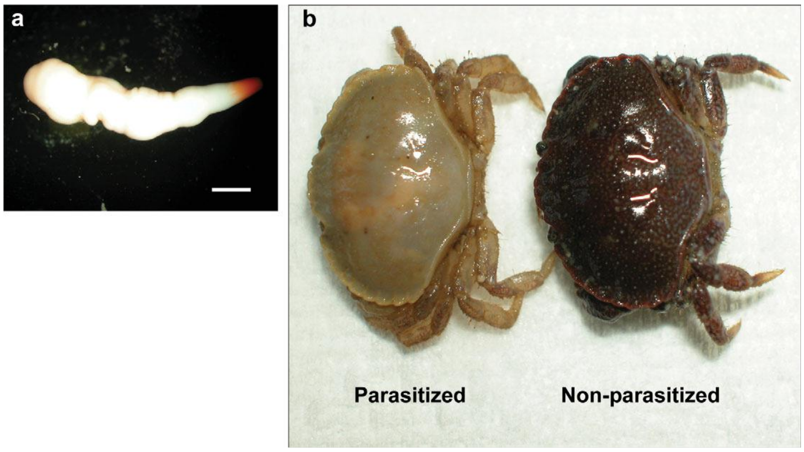


Figure 18 (a) Single *Fecampia erythrocephala,* parasitoid found infecting the haemocoel of juvenile *C. pagurus*. (b) Lighter coloured crab infected with *Fecampia erythrocephala.* Darker coloured crab exhibiting normal pigmentation of *C. pagurus*. Figure taken from (Stentiford 2008).

Other studies such as Bateman & Stentiford (2008) have found other virus' that may be present in juvenile *C. pagurus* populations. This study found a small proportion (5%) of juvenile *C. pagurus* sampled from South Kimmeridge Bay in Dorset to have a bacilliform virus present. The virus is found in the epithelial cells of the hepatopancreas in juveniles between 20mm and 70mm only. No external evidence of disease is present but upon dissection the hepatopancreas appears to have a fine granular matrix in the epithelial cells, which can be detected using a microscope. The effects of this disease are not fully understood but may cause similar physiological starvation as parasitic dinoflagellates that are also found in the hepatopancreas of *C. pagurus* (Stentiford *et al* 2002). This may cause the hepatopancreas to be smaller in size than expected. It may also be a mortality driver, which may affect the size and distribution of the population (Stentiford 2008). Further research into the presence and extent of pathogens and parasites in the juvenile stage of this species particularly in this area will be advantageous to further understand drivers that affect the productivity of the species.

Although using the hepatopancreas as a condition factor is an accurate method of ascertaining the health of crustaceans this method had many limitations. The wet weight was measured, which makes the results incomparable to other studies for example Heath (1970) as they use dry weights. Prior to moulting the individual will allow for the uptake of water, some of this additional water is stored in the hepatopancreas, by measuring the wet weight inaccurate condition factors may be recorded. A difference in water stored in the hepatopancreas between sexes has been seen in *Carcinus maenas* with males holding more water than females (Barrento 2009). Although this has not been described for *C. pagurus*, there may be a bias in composition of the hepatopancreas between the sexes.

4.4 Conclusion

This study successfully used a methodology standardised by Dickinson (2012) to collect abundance data for juvenile C. pagurus from the intertidal area of shores around north Wales for 2013. 2 new sites were identified as hosting a population of juvenile *C. pagurus* expanding knowledge of their presence. Boulders were identified as a parameter possible influencing the abundance of the crabs on the shore due to their sheltering ability which protects individuals from predators and changing environmental parameters.

A mark and recapture study was successfully conducted which revealed the level of movement between the intertidal habitat and the surrounding area. The mark and recapture study also found a tag and adhesive that can be successfully be used in the field on crustaceans, reducing the time required for marking events. The weight of the hepatopancreas was used as a condition factor in an attempt to identify sites that were providing *C. pagurus* with nutrients that allowed them to grow faster there than at other sites. This method appeared to have limitations with the procedure such as measuring the wet weight, however if these were rectified an accurate condition factor could be recorded.

The potential presence of parasites and diseases in the juvenile population is concerning as it could be influencing the survival of an exploited species.

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6.0 Appendices

Site Name	**Location**	**OS Grid references**	**Presence of juvenile C. pagurus**	**Habitat description and accessibility**
Aberdaron	Llŷn peninsula	SH 173 263	No	Mainly Sandy shore, rocky were small pebble patches
Abersoch	Llŷn peninsula	SH 317 283	No	Mainly sandy with rocky outcrops not easily accessible and not resembling a zoned rocky shore.
Abberffraw	Anglesey	SH 353 678	No	Mainly sandy and estuarine, rocky outcrops were mainly bedrock. Access was not good, would have to walk 15 minutes to the shoreline.
Bull bay	Anglesey	SH 429 939	Yes	Mainly pebbled beach good for access, crabs were not found initially but on subsequent trips some were found on a rocky outcrop heavily covered in a fucoid layer.
Criccieth	Llŷn peninsula	SH 499 375	Yes	Sandy habitat with a large boulder section. Good access.
Great Orme	North Wales		Unknown	Not accessible so no observation was done on

				habitat.
Llandudno pier	North Wales		Yes	Boulder shore under the pier with fucoids present. Good access
Llanengan	Llŷn peninsula	SH 283 262	No	Mainly a sandy shore and access was not good.
Mariandyrys	Anglesey	SH 605 819	Unknown	Not accessible so habitat was not observed.
Menai Bridge	Anglesey	SH 556 715	Yes	Under the bridge is a small boulder shore. Good Access.
Y- Swint	Anglesey	SH 517 869	Yes	A rocky shore of both boulders and bedrock. Vehicle access is restricted by local residents, but can walk to the beach in 5 minutes.
Morfa Nefyn	Llŷn peninsula	SH 275 412	No	Rocky outcrops of bedrock Limited access
Porth Colmon	Llŷn peninsula	SH 204 346	No	Rocky shore mainly bedrock. Good access.
Porth Ysgaden	Llŷn peninsula	SH 216 376	No	Small shore with a dominating jettie. Good access.
Ryd - Whyn	Anglesey	SH 299 894	No	Sandy shore with Bedrock outcrops. Good access.
Trefor	Llŷn peninsula	SH 363 468	NO	Mainly Pebble shore with some large boulders. Good access.

Criccieth *   **Menai Bridge** *

Llandudno   **Y- Swint**

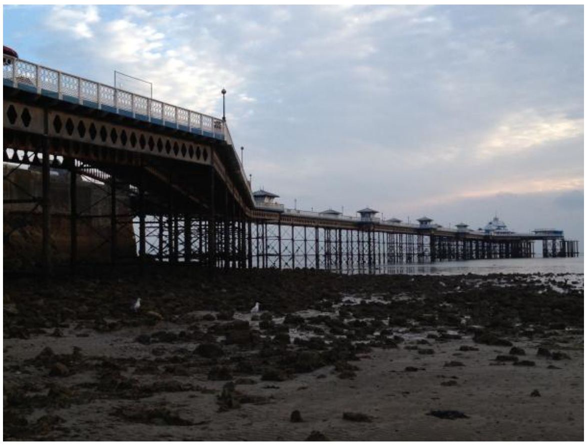

Figure A1 Photographs of sites surveyed during sampling. Sites marked with * were sampled during 2012 also.

Appenidix B. Abiotic and biotic parameters investigated

Table B1 Species of algae, substratum and individual species recorded in $0.25m^2$ quadrats in each times area of the fucoid and laminaria zone.

Environmental parameter measured	Species / physical property measured
% Coverage of algae and barnacles	*Ascophyllum nodosum* *Corallina officinalis* *Chondrus crispus* *Enteromorpha* *Fucus serratus* *Fucus spiralis* *Fucus vesiculosus* *Laminaria digitata* *Palmaria palmata* *Pelvetia* *Ptilota plumosa* *Ulva lactuca* Barnacle sp
% Coverage of substrate	Bedrock Boulders Cobbles Gravel Sand Shell
Abundance of species	*Asterias rubens* *Carcinus maenas* *Galathea squamifera* *Gibbula cineraria*

	Gibbula umbilicalis *Littorina littorea* *Littorina obtusata* *Littorina saxatilis* *Mytilus edulis* *Nucella lapillus* *Patella vulgata* *Pholis gunnellus* *Pilumnus longicornis* *Porcellana platycheles* *Talitrus saltator* *Actinia equina*

Table B2 aperture sizes of sieves used to identify particle size (mm) and phi (ϕ).

mm	phi (ϕ)
16	-4
8	-3
4	-2
2	-1
1	0
0.5	1
0.25	2
0.125	3
0.063	3.99

Appendix C Additional studies

Is-adran Môr a Physgodfeydd /
Marine & Fisheries Division

Llywodraeth Cymru
Welsh Government

Dr Jodie Haig (EFF)
Fisheries and Conservation Science
School of Ocean Sciences
Askew Street
Menai Bridge
Anglesey
LL59 5AB

14th June 2013

Dear Dr Haig,

Re: Dispensation from:

THE PROVISIONS OF COUNCIL REGULATION 850/98 Annex VII

and

THE MARINE AND COASTAL ACCESS ACT 2009 (Commencement No. 1 Consequential, Transitional and Savings Provisions) (England and Wales) ORDER 2010

Thank you for your e-mail on the 10th June 2013. This dispensation grants an exemption from the above legislation in order to retain edible crab below the EU minimum landing size of 130mm and land more than 5 individuals per calendar day between June and September 2013

The aim of this researh is to determine suitable nursery habitats and sample size frequency for juvenile Cancer pagurus using counts of abundance, size and document habitat type. This data will contribute to long term sampling program to establish a recruitment index for the Welsh Cancer pagurus fishery.

Up to 200 crabs may be retained and transported to Menai Bridge School of Ocean Sciences laboratory for analysis.

This letter of Dispensation is to be held by Jodie Haig, Julia Pantin, Harriet Salomonsen and MSc students Michel Hayle and Nikki Heraghty. The letter of Dispensation must be held by anyone of these persons conducting this fieldwork and presented to a Marine Enforcement Officer on request.

Phil Marshall
Phil Marshall
Marine Enforcement Officer.

BUDDSODDWYR MEWN POBL | INVESTORS IN PEOPLE

Is-adran Môr a Physgodfeydd /
Marine & Fisheries Division
Llywodraeth Cymru / Welsh Government

Figure C1 Image of dispensation form to allow the collection of undersized juvenile *C. pagurus* for hepatopancreas dissection and tag retention study.