SR688 The velvet swimming crab (*Necora puber*) fishery in Northern Ireland: a study of populations and welfare to enhance sustainability

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The velvet swimming crab (*Necora puber*) fishery in Northern Ireland: a study of populations and welfare to enhance sustainability

FINAL REPORT

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Queen's University Marine Laboratory

SEAFISH the authority on seafood

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SECTION A: Context

A1. *Executive Summary*

1. Between 2006 – 2009, approximately 230 tonnes of velvet swimming crab, *Necora puber,* were landed each year in Northern Ireland. But in 2010 this declined to around 180 tonnes (AFBI 2013). The fishery has since been in further decline, with only 135 tonnes landed in 2014 (DARD pers. comm.); this has led to concern among local fishermen, government and industry.

2. In 2012, Seafish awarded a £69,600 research grant to Queen's University Belfast (QUB) for a PhD project designed to tackle issues within the velvet swimming crab industry in Northern Ireland. QUB contributed a further £52,386 in terms of supervisory time, training costs and general laboratory costs. An estimated £4,725 was also contributed in-kind by fishermen and other steering group members.

3. Projects outlined in this report were therefore designed in consultation with Seafish and fishermen to be informative to the fishing industry in N. Ireland. The methods and techniques can be applied to other velvet crab populations and crustacean species in the UK and elsewhere.

4. Over two years, a monthly population sampling regime in Strangford Lough and the Irish Sea measured detailed catch demographics. Crabs were significantly larger and heavier in Strangford Lough than the Irish Sea. Brood size was positively correlated with female body size. Catches from both areas had a male-biased sex ratio in the catches; however, this varied seasonally. In autumn (Aug-Oct), male:female sex ratio was 1:1; in winter (Nov-Jan) 2:1 and spring (Feb-April) 3:1 and in summer (May to July) 3:1. Limb loss did not vary among seasons between Portavogie, Portaferry and Whiterock, though limb loss was consistently higher in Ballywalter, with highest numbers of crabs missing limbs found in August-October. In both Strangford Lough and the Irish Sea, higher proportions of berried females were found in spring months (Feb-April); berried crabs were present all year, but mostly absent in June-November.

5. Due to an industry-proposed buy-back scheme, where a voluntary increase in minimum landing size (MLS) would be adhered to and animals between the legal MLS and the enhanced voluntary MLS returned to the sea, with a bounty paid to fishermen for participation in the scheme, a need was identified to mark individual crabs. This is necessary to ensure that such crabs, when returned to Strangford Lough, were not landed before their next moult. The use of external markers as an indicator tool are not widely used in crustacean species due to moulting. Here, marine sealant silicone proved effective as an external marker for velvet crabs and when applied, did not

negatively influence crab behaviour with respect to feeding, mate choice or aggression.

6. Stress can lead to higher crab mortalities, and this research investigated two areas of potential stressors. Stress from air exposure remains a concern for fishermen as they witness higher catch-mortality on fishing days where crabs remain out of water for long periods, particularly on windy days. Experimental crabs were exposed to varying levels of air exposure, and haemolymph (blood) removed at intervals to monitor physiological indicators of stress. Raised glucose and lactate levels indicated that full air exposure, and air exposure but with seaweed cover, were equally stressful. Welfare of berried female crabs was also investigated. Berried females are currently legal to land. In assessing handling of berried females, gentle handling did not impact the behaviour of berried females, or have a negative impact on brood size, but negative effects appeared with rougher handling. In a physiological experiment, berried crabs were handled for one minute and haemolymph removed at time intervals to determine recovery period after handling stress. Short-term stress was indicated by elevated lactate levels.

7. A new method published in 2012 for ageing crustaceans, that of counting growth bands on the eyestalks, was trialled with velvet crabs. We were able to remove, decalcify and dehydrate eyestalks, embed them in resin and paraffin, and produce 1.5-7 µm sections with a microtome. Structures were inspected under a microscope and potential growth bands identified. Further work on ageing in this and other crustacean species could resolve age/size relationships for future management strategies.

8. As the project developed, DARD looked to our results for guidance on decisions and policy and ensure sustainable management of the fishery. Our science-based conclusions and recommendations for policy and practice are as follows:
   a. Closed seasons for berried females between December and April; closed periods should be site/population specific or alternatively a complete year-round ban on the landing of berried crabs;
   b. The use of marine sealant silicone as a marker is advised in management strategies, such as to aid in buy-back schemes if minimum landing sizes were increased;
   c. Air exposure and handling of crabs should be minimised to reduce mortality and stress; and
   d. Further ageing studies are required to aid management.

A2. *Introduction*

Historically, management of fisheries has often been unsustainable (Pauly *et al.* 2002). Natural variation in populations can mask the effects of over-exploitation, which may not be acknowledged until severe, and sometimes irreversible (Ludwig *et al.* 1993), due to poor detection skills (Dulvy *et al.* 2003). Detailed scientific analyses can be used to identify important fishery trends (Pauly and Palomares 2005), and data on populations and individuals can be used to underpin strategies for industry and policy recommendations and leglisation.

The velvet swimming crab (*Necora puber*) is traditionally consumed by southern European nations such as France and Spain. A sudden decline in fisheries in the 1980s due to over-exploitation and disease (Wilhelm and Mialhe 1996) led to the commercial development of the fishery in the UK and Ireland (Fahy *et al*. 2008). Further, *N. puber* fisheries in France have been in decline since 1984 due to infection with the dinoflagellate *Hematodinium* sp. (Wilhelm and Mialhe 1996). This is well known for causing 'Pink Crab Disease' in the brown crab (*Cancer pagurus*) as the meat and haemolymph appear pink in colour, causing the meat to taste bitter. The *N. puber* fishery in Northern Ireland could increasingly be a valuable fishery for international markets if managed for sustainable exploitation.

Though previously considered a pest species (MacMullen 1983), the velvet crab is now a commercially significant export species in the UK and Ireland. Within the pot fishery, *N. puber* is taken mainly as a bycatch as it is associated in areas with brown crab (*Cancer pagurus*). *Necora puber* has a greater commercial significance in the Irish Sea as there are lesser quantities of brown crabs, and thus are targeted here (Fahy *et al.* 2008). The commercial value of *N. puber* landings in Northern Ireland peaked in 1995 at £24,348 and this has since declined to £5,812 per annum in 2003 (Roberts *et al.* 2004). Between 2006 – 2009, approximately 230 tonnes of *N. puber* were landed per year, with a decline to around 180 tonnes in 2010 (AFBI 2013), further declining to 135 tonnes in 2014 (DARD pers. comm.).

These declines have led to concern among local fishermen, industry and fishery managers. Due to the relatively recent development of the fishery in Northern Ireland, little is known about population parameters such as sex ratios or reproductive habits of velvet crabs in important Northern Irish fishing grounds. A report commissioned by DARD in 2011, "*The Northern Ireland Brown Crab Strategy*", highlighted the need for research in the velvet crab fishery due it being a mixed fishery. To secure the long term sustainable exploitation of *N. puber,* the understanding of population status in local fishing grounds is a priority.

This report details projects undertaken during the course of a three year PhD research project as outlined in our Seafish Industry Project Fund Proposal, with amendments made as agreed by the steering group. Agreed outputs were:

- to provide information on two populations of velvet crabs, those of Strangford Lough and the Irish Sea;
- to assess body size/mass, brood sizes, breeding seasons and sex ratios; and
- to investigate stress due to air exposure and handling.

Additional studies were brought forward by the steering group:

- to determine a marker suitable for tagging in a stock enhancement scheme, and
- to adapt a newly developed method to directly determine age in velvet crabs.

SECTION B: Research results

B1. ***Population assessment of Strangford Lough and Irish Sea velvet crabs***

B1.1. Project background

Due to the relatively recent development of the velvet crab fishery in Northern Ireland, little is known about the current population status and structure. This research investigated currently unknown population parameters, such as seasonality of reproduction, sex ratios and limb loss in fisheries on both Strangford Lough and the Irish Sea coasts of the Ards peninsula. These baseline data may then be used as a basis for management and future policy decisions.

There are currently limited protection measures in place for velvet crabs, thus impacts on vulnerable crabs are a key concern among fishermen, such as soft or berried crabs which are currently legal to land. In Shetland, closed seasons during the moulting and reproductive period protects soft crabs from stress caused by handling or air exposure (Henderson and Leslie 2006).

This research aims to explain when the berried velvet crab season occurs in fisheries located in Strangford Lough and the Irish Sea (east and west of the Ards Peninsula), as it is imperative that the landing of berried crabs is controlled, to ensure minimal disturbance which will ultimately enhance recruitment to the population.

B1.2. Methods

Field surveys of abundance and population structure were piloted in December 2012, and carried out fully between March 2013 – March 2015. We conducted pot sampling with fishermen at four sites in both Strangford Lough (Whiterock and Portaferry) and the Irish Sea (Ballywalter and Portavogie), see Figure 1. Crab gear was supplied by Gaelforce, Cavanagh nets and Shields creels. Data were collected via a logsheet (Appendices 1 and A2). A 0.5g subsample of eggs were removed from 21 females and brood size estimated.

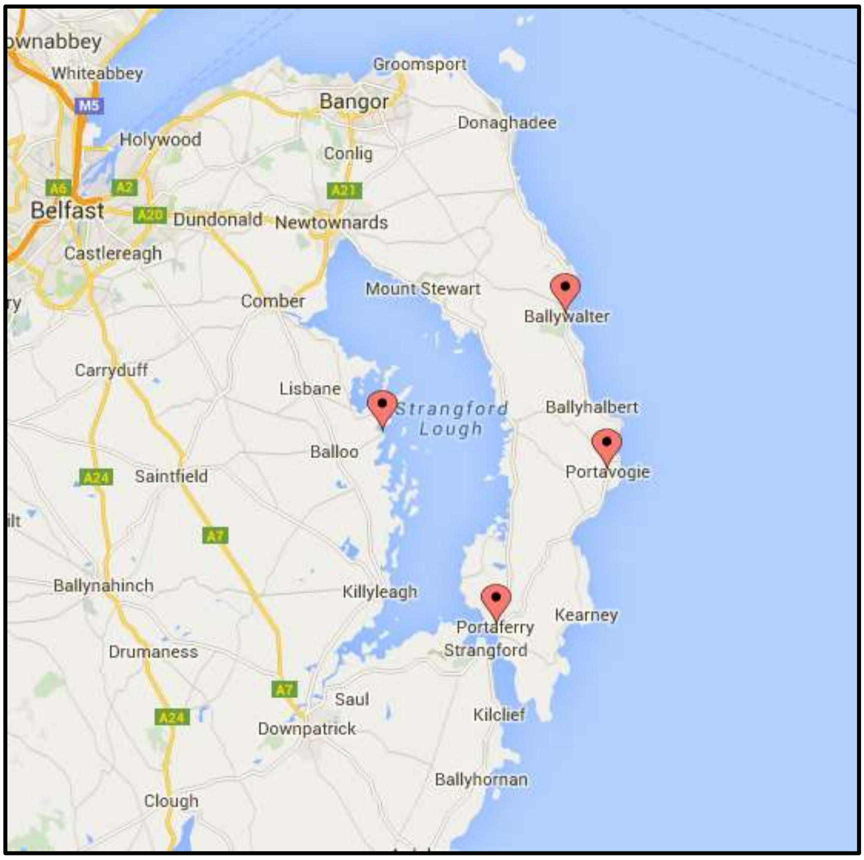


Figure 1. Location of our four study sites: in Strangford Lough, Whiterock and Portaferry; in Irish Sea, Ballywalter and Portavogie.

B1.3. Results

B1.3.1.Morphometrics

- In total, 3623 crabs were caught during the sampling period.
- Crabs ranged in carapace width (CW) between 31mm and 104mm (mean CW Portavogie=70mm; Ballywalter=71mm; Whiterock=76mm; Portaferry=72mm) (Fig. 2a – 2d)
- Crabs ranged in body mass between 11g and 287g (mean mass Portavogie=97g; Ballywalter=100g; Whiterock=129g; Portaferry=107g) (Fig. 3a – 3d).
- Generally heavier, larger animals were landed at the Strangford Lough sites compared to the Irish Sea sites, with the largest and heaviest crabs found in the north of Strangford Lough at Whiterock.
- Males were significantly larger and heavier (mean CW = 74mm, mean mass = 119g) than females (mean CW = 70mm, mean mass = 90g).

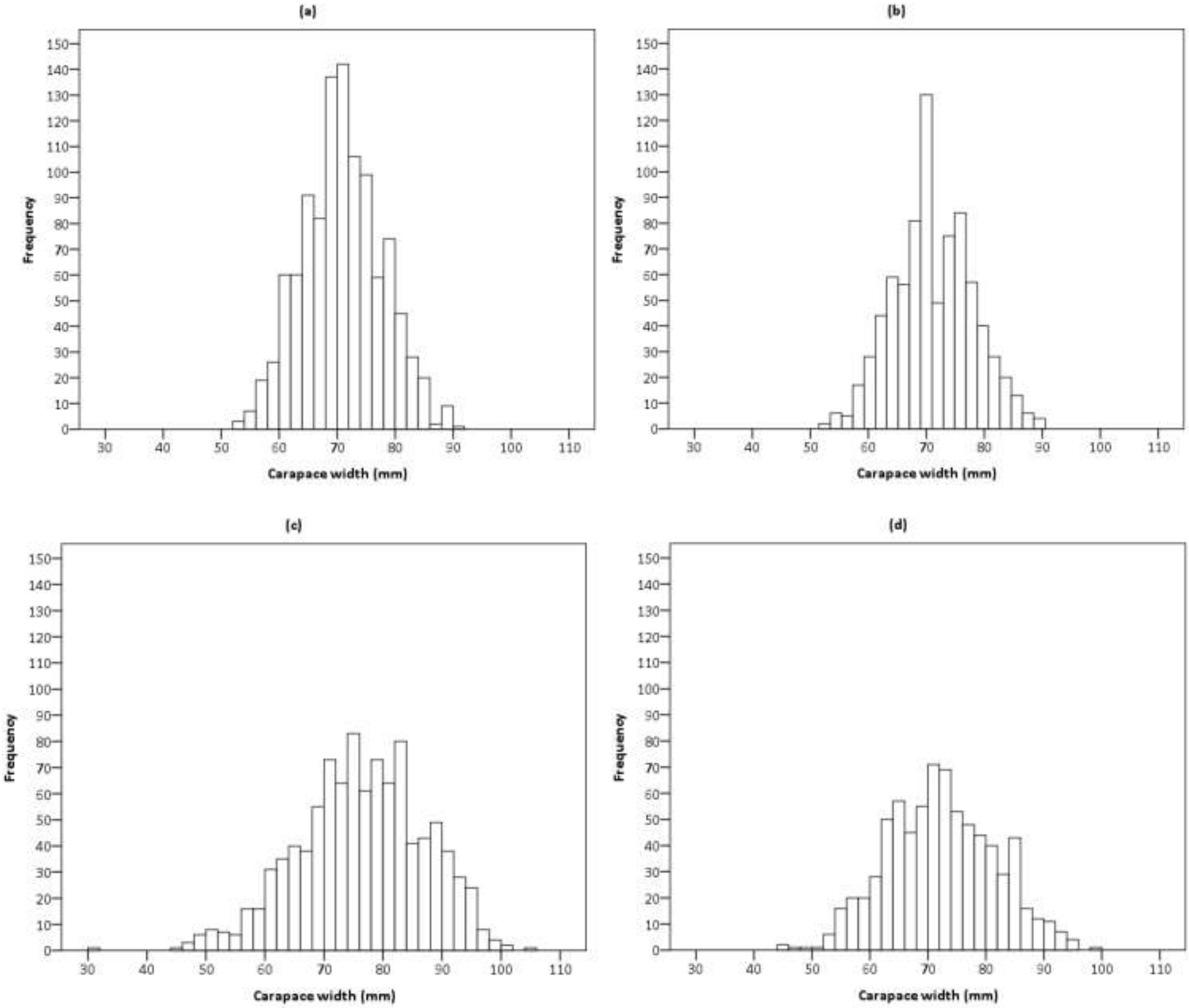


Figure 2. Frequency distributions of carapace width (mm) in: two sites in the Irish Sea: a) Portavogie; b) Ballywalter; and two sites in Strangford Lough: c) Whiterock; and d) Portaferry.

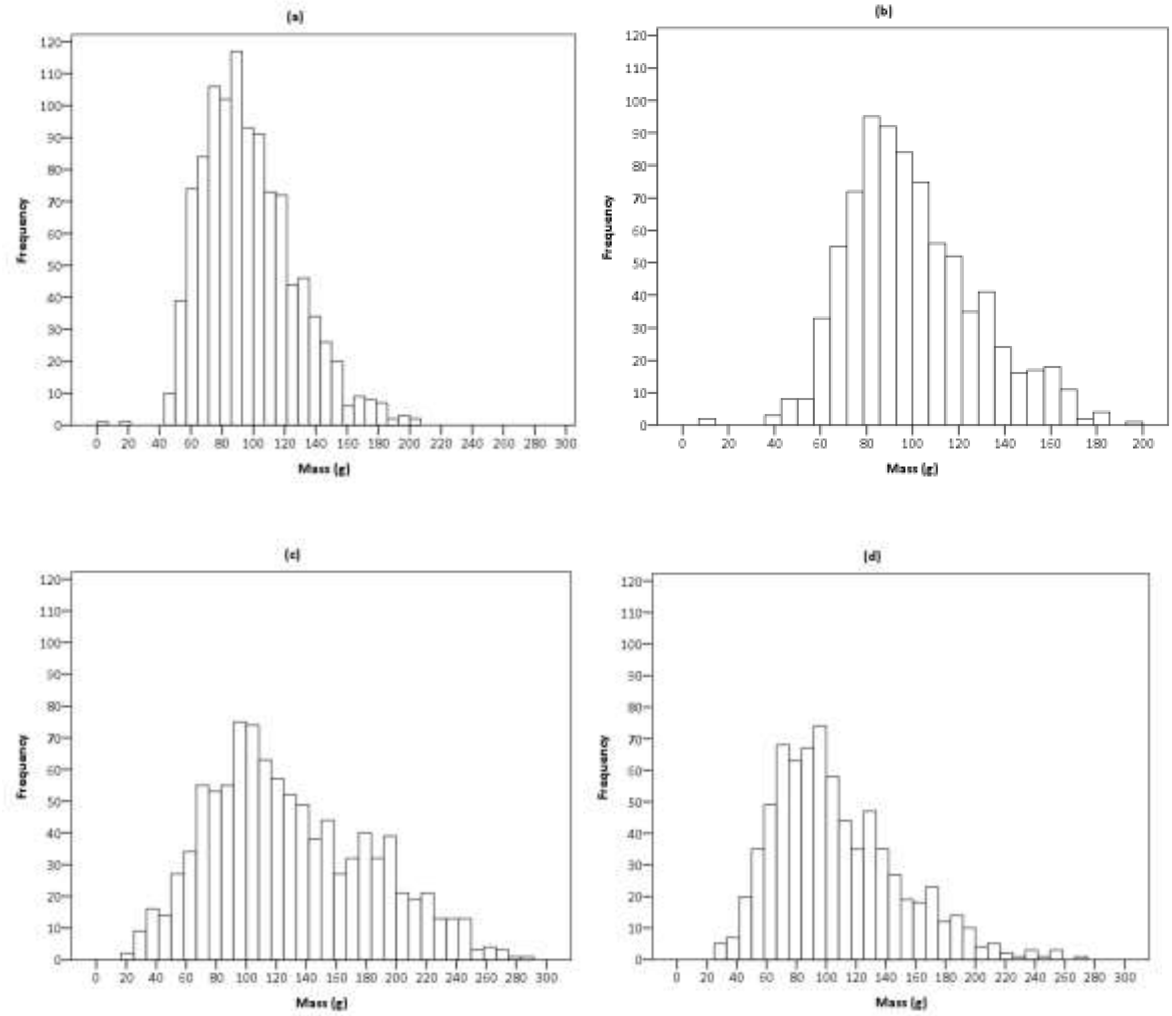
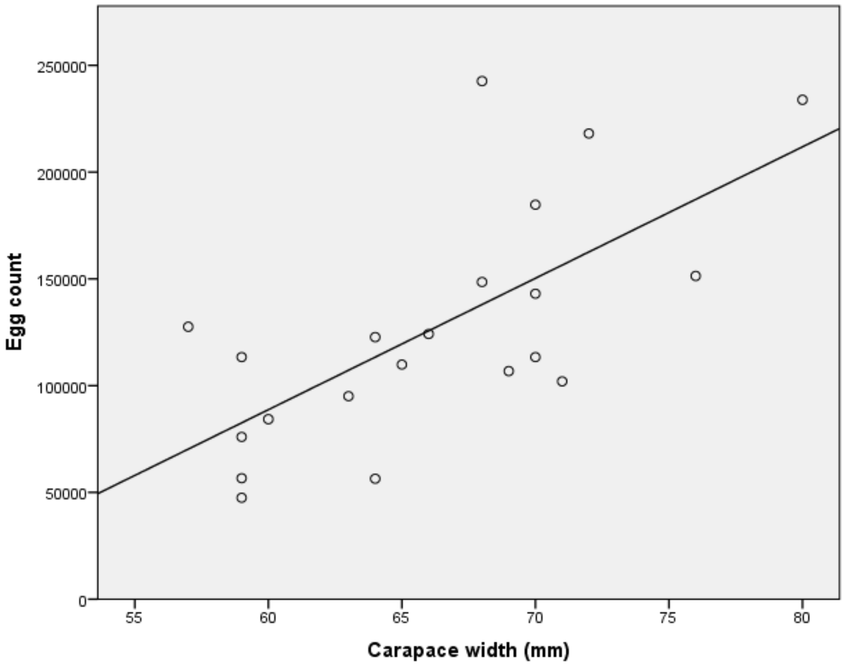


Figure 3. Frequency distributions of crab mass (g) in: two sites in the Irish Sea: a) Portavogie; b) Ballywalter; and two sites in Strangford Lough: c) Whiterock; and d) Portaferry

B1.3.2. Brood sizes

Berried females, ranging in size from 59mm to 80mm, had an estimated brood size of between 48,000 – 243,000 eggs. Larger berried females had a larger brood size (see Figs. 4a, b).

a)



b)

Figure 4. a. Relationship of berried female carapace width to size of egg mass. **b.** Egg sample taken from berried female during egg counts (late developmental stage).

B1.3.3. Sex ratios

Ballywalter (Irish Sea), Portavogie (Irish Sea), Whiterock (Strangford Lough) and Portaferry (Strangford Lough) all showed a male-biased sex ratio in catches. Catch generally consisted of a larger number of males, though the ratio of males:females differed throughout the year (Table 1, Fig. 5b).

Table 1. Seasonal change in sex ratios from all study sites

Months	**Male:Female sex ratio**
Spring (Feb-Apr)	3:1
Summer (May-Jul)	3:1
Autumn (Aug-Oct)	1:1
Winter (Nov-Jan)	2:1

B1.3.4. Limb loss

One in three crabs landed were missing one or more limbs. There was no apparent seasonal pattern of limb loss between sites. Portavogie, Portaferry and Whiterock had similar numbers of crabs missing limbs throughout the year, though limb loss was consistently higher at Ballywalter, with peak limb loss found in Autumn.

Table 2. Percentage of crabs missing limbs at each site throughout the year

	Season			
	Spring	**Summer**	**Autumn**	**Winter**
Site	**Feb-Apr**	**May-Jul**	**Aug-Oct**	**Nov-Jan**
Irish Sea				
Portavogie	24	18	19	29
Ballywalter	35	37	57	30
Strangford Lough				
Whiterock	17	16	12	21
Portaferry	24	29	12	20

B1.3.5. Berried seasons

The seasonal change in proportions of berried females caught was broadly similar between Strangford Lough and the Irish Sea, however, some differences (eg in Autumn; see also Appendix 3) suggest that any closed seasons should be site/population specific. The majority of berried females were caught in the spring months (February - April) in both Strangford Lough and the Irish Sea, with the lowest proportions found from June to November (Fig. 5a-b). Over 35% of all females caught during winter and spring were berried.

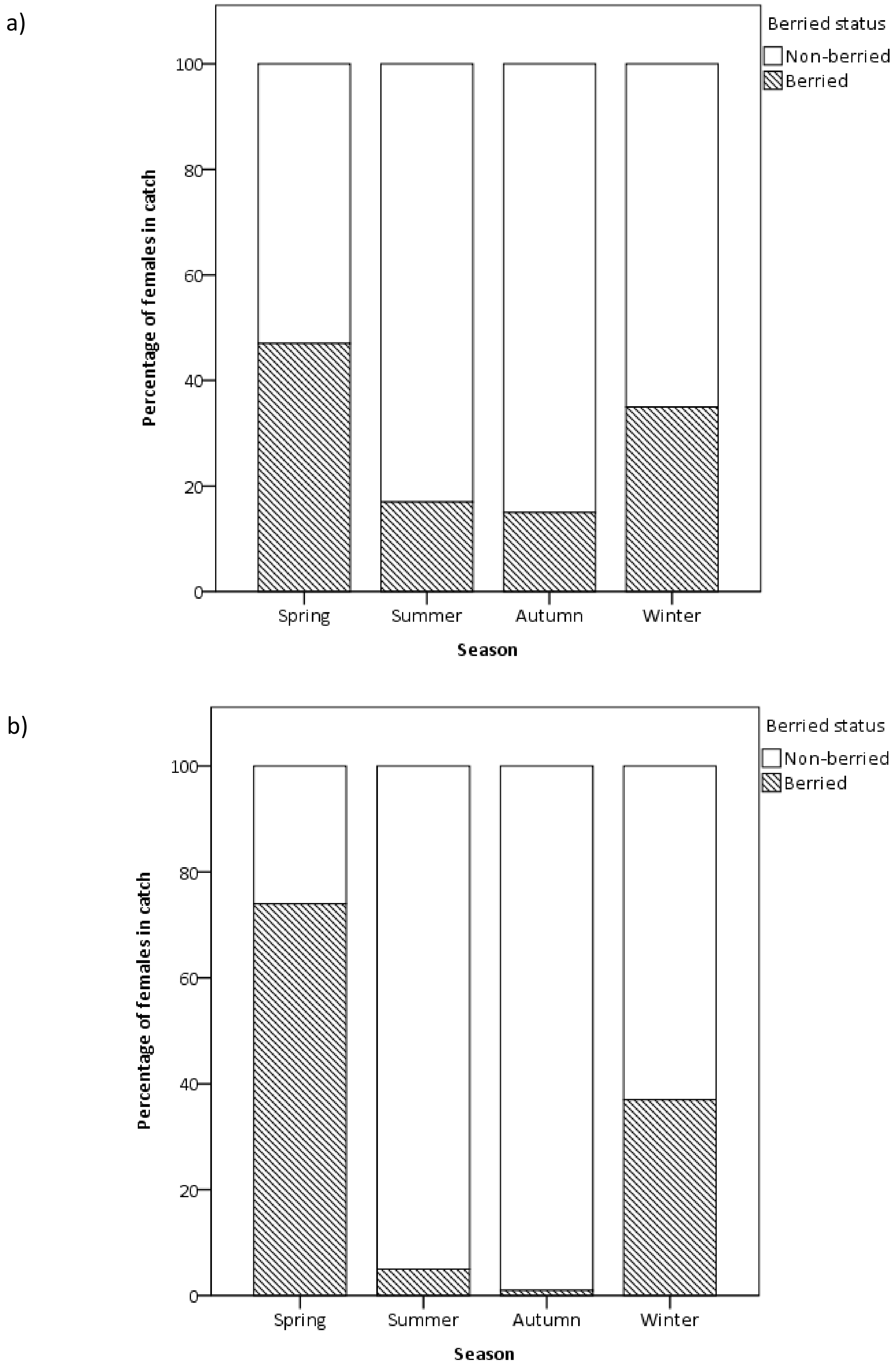


Figure 5. Percentage of females in catch made up of berried females seasonally in a) Strangford Lough; and b) the Irish Sea

B1.4. Discussion

Anecdotal reports had indicated that velvet crabs were larger in Strangford Lough than in the Irish Sea; here, we present evidence-based research to confirm this. Crabs caught in Strangford Lough were consistenly larger (in terms of carapace width and body mass) than crabs caught in the Irish Sea (Figs. 2 and 3). Male crabs were also consistently larger than females, similar to other crustacean species (Warner 1967, Conan and Comeau 1986, Hill 1975, Paul 1992, Baeza *et al*. 2013).

The male-biased sex ratios of caught crabs likely reflects differential catchability of the sexes, rather than different population sex ratios. These results likely reflect the different behaviour of males and females, in that female crabs are less likely to enter pots than males, as seen in the edible crab (*Cancer pagurus*) during reproductive seasons (Howard 1982). Catchability of the southern rock lobster (*Jasus edwardsii*) varies seasonally and with sex (Ziegler *et al.* 2003). Similarly, each site investigated here had seasonal variation in catch, being predominately male biased throughout the year, with the exception of autumn when mating period occurs. Fewer females were caught throughout the spring and summer, which is beneficial to the fishery as many females are berried in the spring. However, females are also berried during late winter, when slightly more females were caught at a 2:1 male:female ratio. Limb loss did not vary significantly among seasons between Portavogie, Portaferry and Whiterock, though was consistently higher in Ballywalter. High levels of injury may typically be seen in crustaceans, with no correlation between sex or size (Juanes and Smith 1995).

Notching the tail of berried lobsters and returning the animal to the sea is common practice throughout the UK and Ireland (AFBI 2003). These known breeding females boost egg production and enhance recruitment to the population as they are protected and must be returned to the sea when caught. Protection of berried females has shown increased egg production in fisheries (Telsnig 2013). Similar protective legislation of berried female velvet crabs would enhance recruitment to the population, as females would be returned to the sea and allowed to breed during the winter.

These results provide baseline data about the current composition of catch in Strangford Lough and the Irish Sea, which will allow for better management strategies to be put in place, with particular regard to berried females (see B5). For example, any increase in the minimum landing size of crabs would enhance possible recruitment to the population, due to larger females having a larger brood size (Fig. 4a).

B1.5. Conclusions

- **Crabs caught in Strangford Lough were consistenly larger (in terms of carapace width and body mass) than crabs caught in the Irish Sea.**
- **Male velvet crabs were consistently larger than females.**
- **Larger females carry more eggs.**
- **There was seasonal variation in catch composition, with a greater proportion of males in the the catch, except in autumn, when mating occurs and the ratio of males:females was 1:1.**
- **Limb loss did not vary significantly among seasons between Portavogie, Portaferry Ballywalter and Whiterock, which showed similar numbers of crabs missing limbs throughout the year. Limb loss was though consistently higher in Ballywalter, with peak numbers in autumn months (August-October).**
- **The majority of berried females were caught in the spring months (February - April) in both Strangford Lough and the Irish Sea, with the lowest proportions found from June to November.**

B2. *The influence of external markers on Necora puber behaviour*

B2.1. Project background

The potential increase in minimum landing size (MLS) of *N. puber* from 65mm to 80mm in Strangford Lough has scope to enhance *N. puber* populations by allowing females to reproduce for a greater number of seasons before being a commercially landable size. However, an increase in MLS will result in a loss for fishermen due to a time lag between size generations. To mitigate financial losses for fishermen, a buy-back scheme may be introduced, whereby crabs of a previously landable size (65-79mm) are purchased from fishermen but returned to Strangford Lough. To prevent fishermen being paid multiple times for the same animal, an external marker could be used to distinguish them as a "previously landed animal" in the same way as a V-Notch is used to mark female lobsters. This marker must withstand being attached to the animal for up to a year before moulting occurs, after which they may be large enough to be landed.

This study aimed to trial potential external markers that could be incorporated into the buy-back scheme. Behavioural experiments were carried out to examine if *N. puber* individuals are negatively affected by the presence of the marker with regards to response to the marker itself, feeding, mate choice and aggresion. These experiments will thus allow an informed decision to be made about the use of external markers in the velvet crab fishery.

B2.2. Methods

B2.2.1. Testing potential markers for tagging crabs

Three adhesive materials were trialled as potential markers: aerosol paint, Milliput and marine sealant silicone. Milliput remained attached to the carapace for up to one week, while the aerosol paint lasted for up to six weeks. Silicone proved to be the most effective method of marking crabs, lasting the length of the trial (4 months) and thus was used in all further experiments.

B2.2.2. Preference-avoidance behaviour towards the tag material

To test the general response behaviour of crabs to the tag itself, blue piping (12.5cm diameter x 18cm length) was placed at either end of an 85.5 litre brightly lit glass tank (75cm length x 30cm width x 38cm height) to act as shelter, with an open area between the shelters. A layer of stones and sand on the floor of the tank ensured that the shelters remained in place and provided a more natural environment for the crabs. The inside of one shelter was covered with a layer of the tag of choice, marine sealant silicone. Individual crabs (sample size = 18, carapace width range = 64 - 80mm) were placed in the centre of the tank and the amount of time in visits to the two shelter types was recorded over a 10 minute period to examine the preference or avoidance of the tagged shelter. To control for bias of the crabs to a particular side of the tank, each crab participated in four trials. After two trials, the shelters were swapped over to the opposite side of the tank. This gave a total of 72 trials.

B2.2.3. Feeding rate of tagged and untagged crabs

Crabs (sample size = 63, carapace width range = 54 – 90mm) were randomly selected to be tagged with silicone and placed overnight in individual 3.5 litre plastic tanks (20cm length x 12cm width x 15cm height) supplied by a seawater source. Non-tagged crabs (sample size = 20) were handled similarly to the tagged crabs (sample size = 43) to eliminate handling bias. To minimise visual disturbances, a viewing window was created by covering three sides of each tank in black plastic and red plastic film was placed over lighting.

Crabs were offered pre-weighed pieces of Atlantic herring (*Clupea harengus*) and left to feed for three days. Uneaten fish was removed from the tanks, weighed and then dried at 65^{o}C for one week to determine dry weight.

B2.2.4. Mate choice

Visual disturbances were minimised using the methods as above. Male crabs (sample size = 30, carapace width range = 64 - 75mm) were placed overnight in individual 20 litre plastic tanks (36cm length x 23cm width x 23cm height) supplied by a seawater source. Females (n = 60, CW range = 54 – 73mm) were randomly selected to be tagged with silicone. A tagged and a non-tagged size-matched female were introduced to the tank with each male and behaviour recorded over a five hour observational period. Males were assessed as to which female was chosen on the basis of mate guarding behaviour.

B2.2.5. Aggression between other crabs

Visual disturbances were minimised using the methods as above. Size-matched crabs (sample size = 28, carapace width range = 65 – 79mm) (one tagged, one untagged) were placed at either end of a 85.5 litre glass tank (75cm length x 30cm width x 38cm height) separated by an opaque barrier. After a 15 minute acclimatisation period, the barrier was removed and interactions of increasing intensity between the two crabs were recorded.

1. Neither crab displays or strikes; one crab retreats
2. One crab displays and may strike the other; one crab retreats
3. Both crabs display and may strike each other; one crab retreats

B2.3. Results

- Crabs did not show any preference/avoidance for either the tagged or untagged shelter (Fig. 6).
- There was no difference in the amount of fish that tagged and untagged crabs ate (Fig. 7).
- Male crabs showed no statistically significant mate preference with respect to female crabs based on tag status, with 47% choosing the tagged female, and 53% the untagged female.
- On most measures of aggression, tagged and untagged crabs showed little difference (Fig. 8).

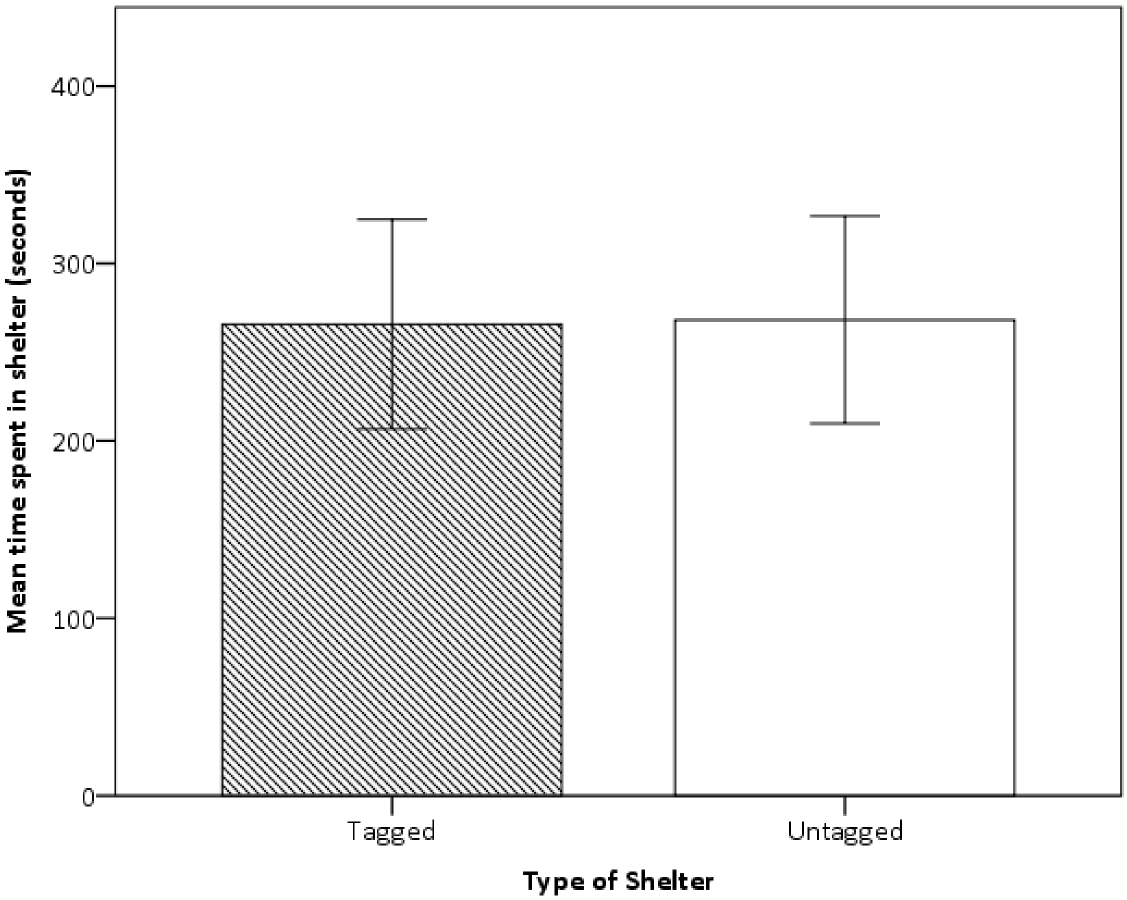


Figure 6. Mean length of time crabs spent in tagged and untagged shelters (seconds)

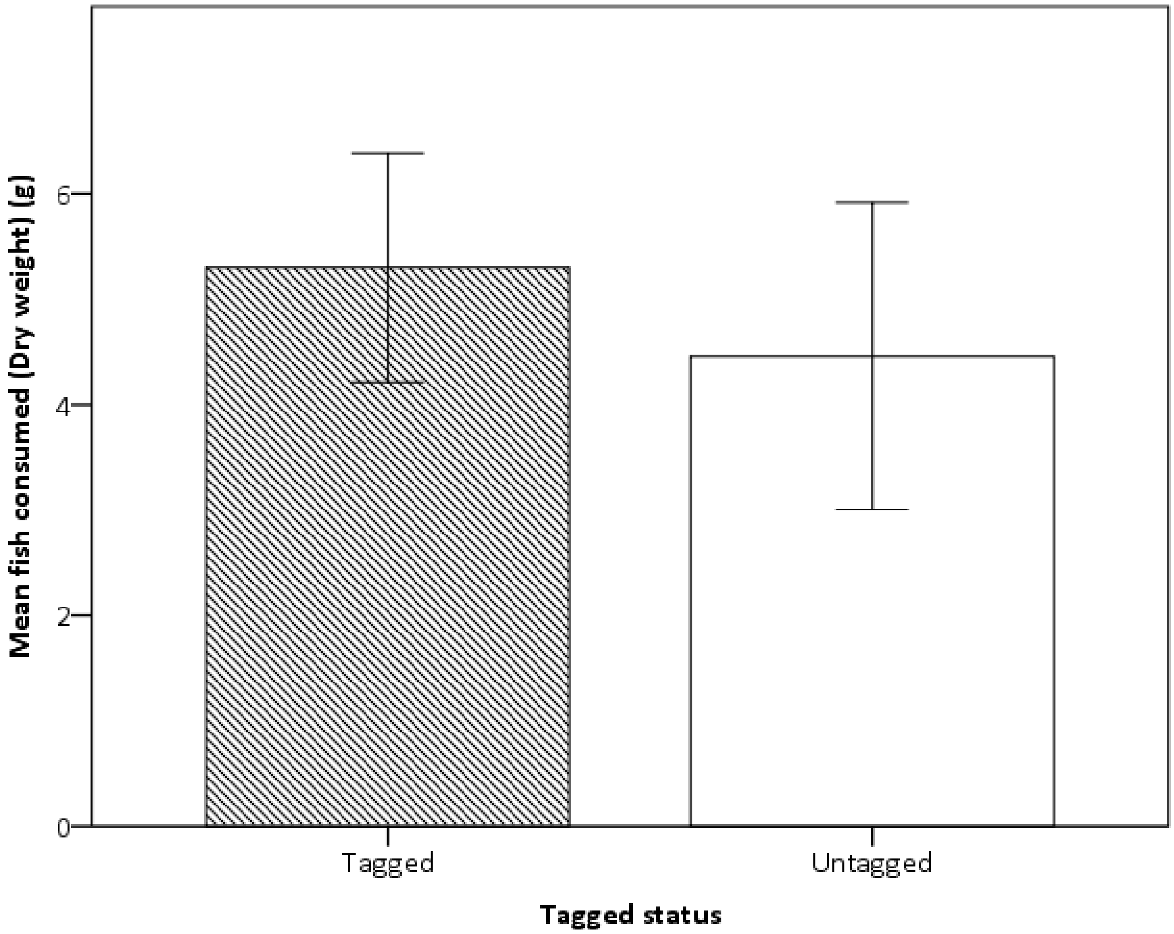


Figure 7. Mean mass of fish consumed (Dry weight) (g) (± S.E.) of tagged and untagged crabs

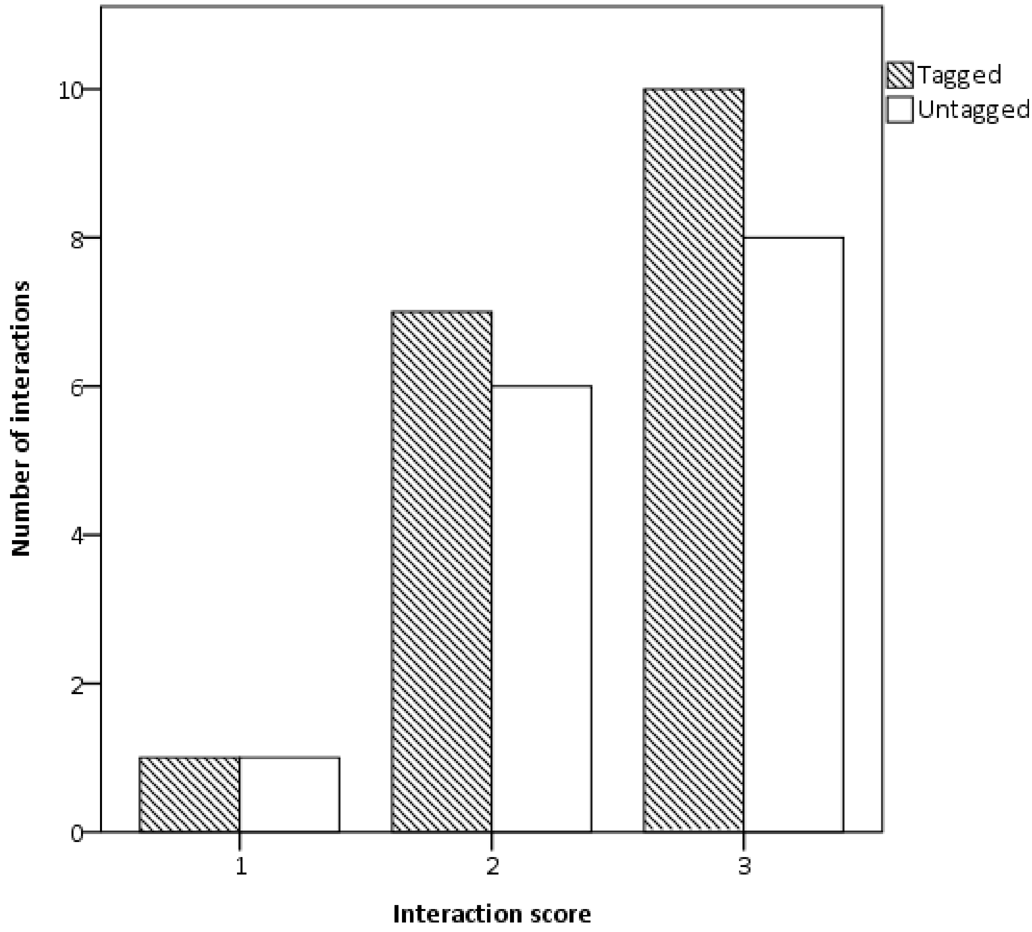


Figure 8. Number of interaction types won by tagged and untagged crabs

B2.4. Discussion

Overall, the presence of silicone as an external marker did not result in any significant behavioural changes in velvet crabs in terms of:

- Shelter choice: crabs spent a similar amount of time in the tagged and untagged shelters, indicating no preference/avoidance behaviour towards the tag material.
- Feeding rate: tagged and untagged crabs ate a similar amount of fish during experimental trials.
- Choice of mate: tagged and untagged female crabs were both mate-guarded by males, and were shown no preference based on tag status.
- Aggression between crabs: tagged and untagged won similar numbers of fights.

External markers are used widely throughout marine species for tagging, and are a valuable identification tool (Baker *et al.* 2014, Brian *et al.* 2014, Goldbogen and Meir 2014, McIntyre 2014). The use of tagging in crustaceans is limited due to the loss of any external marker when the animal moults; however, the nature of the proposed stock enhancement scheme means this would not be an issue. If crabs are caught and tagged, it is likely that they will have a reached the new landable size by their next moult, where the tag will no longer be attached to the carapace. Thus, marine sealant silicone is recommended as a means of marking velvet crabs for a potential fisheries enhancement scheme.

B2.5. Conclusions

- **Marine sealant silicone proved to be the most long-term and effective method of marking crabs, and had no affect on a range of crab behaviours such as shelter choice, feeding rate, choice of mate and aggression.**
- **Marine sealant silicone is therefore recommended as a means of marking velvet crabs for a potential fisheries enhancement (buy- back) scheme.**

B3. *Assessment of air exposure and handling on crab stress and welfare*

B3.1. Project background

Within the Northern Ireland pot fishery, velvet crabs are caught as an export species and transported live (as a guarantee of quality) to the Mediterranean. High mortality during transport devalues the initial catch and results in more crabs being caught to compensate for those that will die *en route*. Velvet crabs are difficult to keep alive as they are particularly sensitive to stress, which may be in the form of extreme temperature, rough handling or dessication.

Velvet crabs often become entangled in fishing gear, and force is required to remove them from creels. This can result in injury, such as limb loss or damage to the carapace. Limb loss may result in female velvet crabs carrying abnormally low brood sizes (Norman and Jones 1993). Rough handling and entanglement may therefore have more damaging consequences for berried females than other crabs in a less vulnerable state, as they are still currently legal to land.

Levels of crustacean hyperglycaemic hormone (CHH) increase during periods of stress. This hyperglycaemic response in crustaceans results in elevated glucose and lactate concentrations in the haemolymph (Abramowitz *et al.* 1944) and can be used as an indicator of stress and welfare. By monitoring changes in haemolymph, the role of CHH as a measurement of stress has been applied to a range of crustacean species (Zou *et al* 1996, Chang *et al* 1999). From this, recommendations can be made to industry to mitigate the effects of stress and thereby reduce mortality.

B3.2. Methods and results

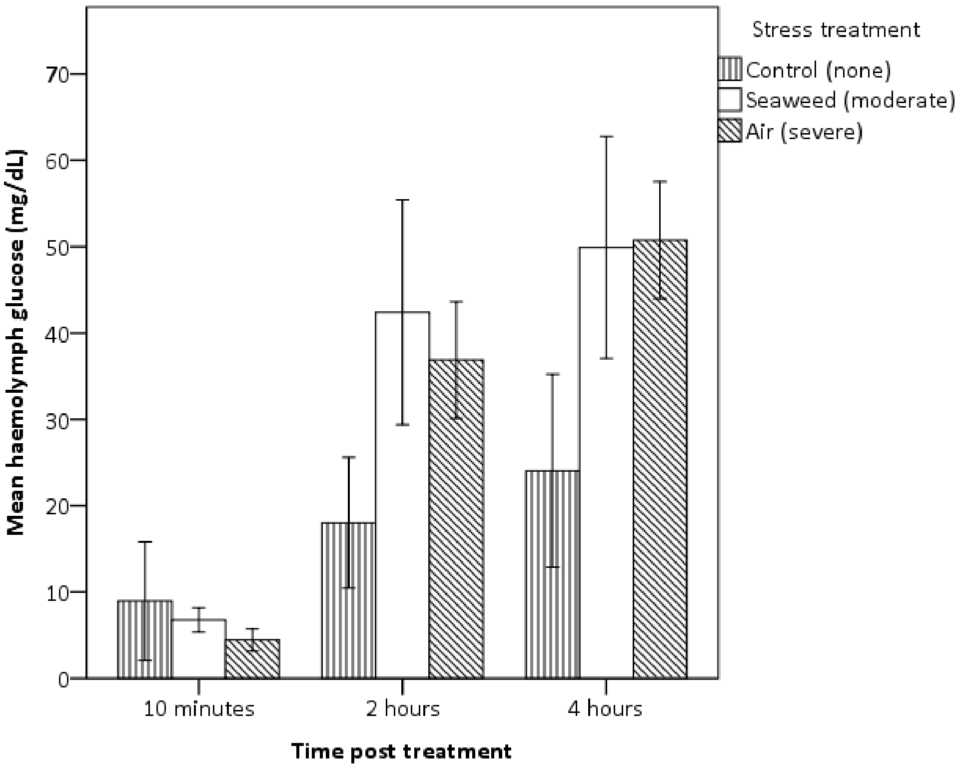
Experiment 1: Physiological effect of air exposure stress on velvet crabs
Crabs (sample size = 30, carapace width range = 66 – 82mm) were placed in individual 3.5 litre plastic tanks (20cm length x 12cm width x 15cm height) and randomly chosen to experience a level of air exposure stress: 1) none (control), where the animal was kept on seawater flow through; 2) moderate, where the animal was covered in wet seaweed; and 3) severe, where the animal remained in air (Figure 9). Haemolymph was removed at 10 minute, 2 hour and 4 hour intervals to monitor the change in lactate and glucose levels using assay kits purchased from Cambridge Bioscience (glucose) and Sigma-Aldrich (lactate).

Figure 9. Levels of air exposure stress; left to right: (1) no stress (crab in seawater); (2) moderate stress (crab covered in damp seaweed); and (3) severe stress (crab in air)

Results

Glucose and lactate were significantly higher in crabs after 2 hours and 4 hours exposure to treatments 2 (seaweed) and 3 (air) (Figure 10a and 10b). The amount of glucose and lactate in the haemolymph was not significantly different between treatments 2 and 3; thus, crabs subjected to any sort of air exposure exhibited a similar stress response.

a)



b)

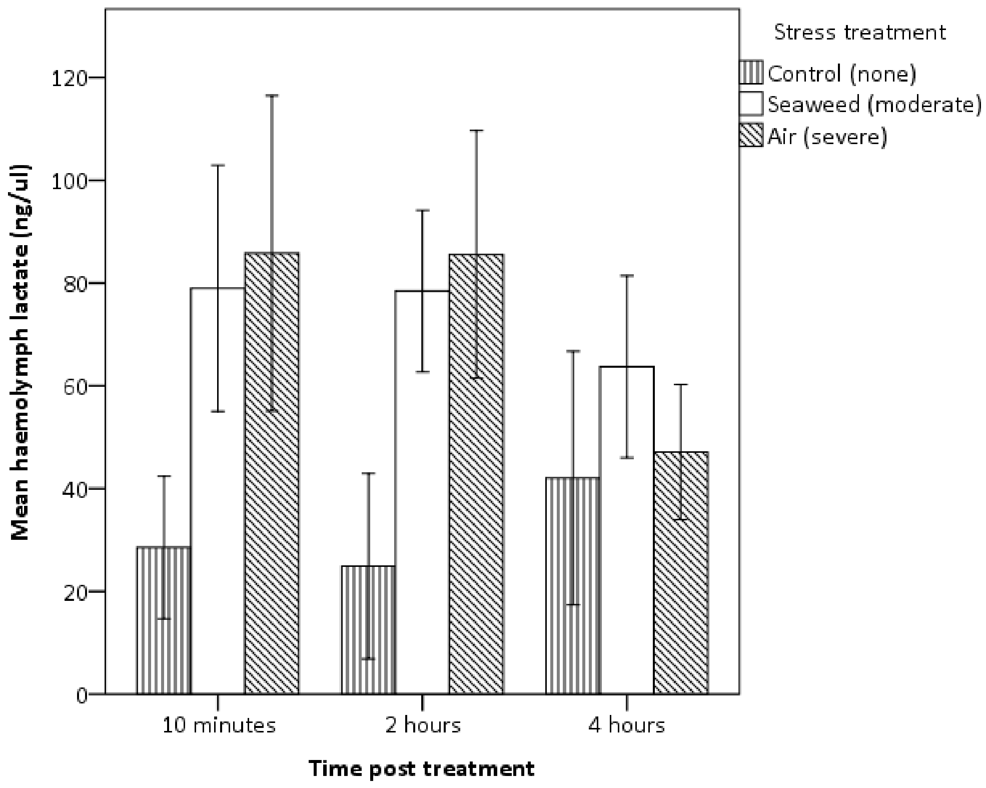


Figure 10. Mean (± S.E.) concentrations of: a) haemolypmh glucose; and b) haemolymph lactate after exposure to air exposure stress.

Experiment 2: Behavioural assessment of handling on berried females

When crabs are landed on fishing vessels, fishermen place crabs the correct way up to alleviate stress. In this assessment, the relationship between handling severity and ability to return to a comfortable, natural position was investigated. Crabs were randomly chosen to experience a level of handling: 1) no handling, 2) mild handling, 3) moderate handling or 4) severe handling. Crabs were handled for 20 seconds, placed on their backs and timed to see how long it took them to correct themselves, either in the presence or absence of other crabs. One week after handling treatment, a 0.5g subsample of eggs were removed from the female and brood size estimated.

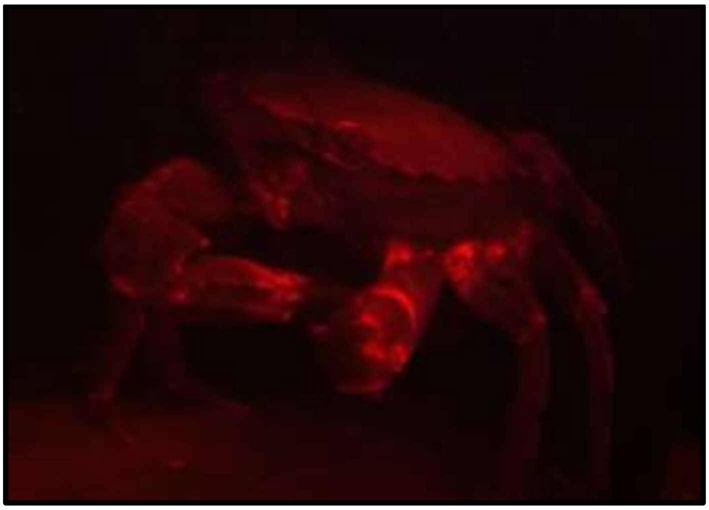


Figure 11. Cheliped probing in a berried female crab; initial behaviour observation study.

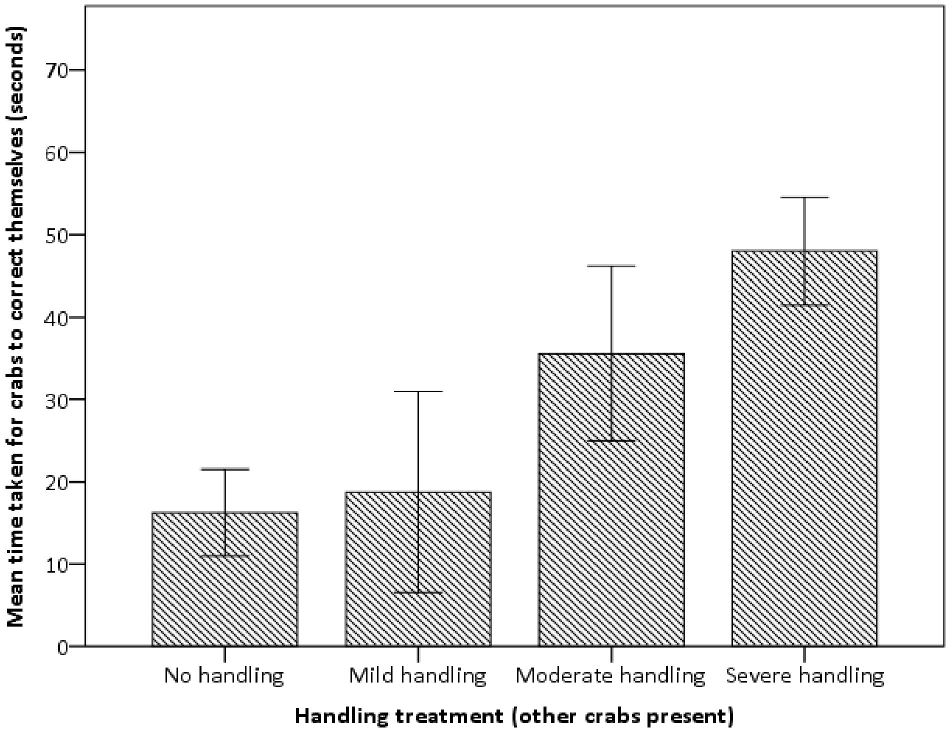
Results

<u>Other crabs present:</u> As handling severity increased, crabs took longer to correct themselves; however, there were no differences between the no handling and mild handling group (Fig. 12a). From a fishery perspective, these results highlight that berried females caught in creels but handled gently exhibit the same behavioural response as crabs that were not handled at all.

<u>Other crabs absent:</u> As handling severity increased, crabs took longer to correct themselves, although most crabs failed to turn over (Fig. 12b). Results of both trials indicate that crabs handled more severely take longer to correct themselves to their natural position, however, the motivation to turn over is stronger when other crabs are present.

<u>Brood size:</u> Egg counts showed no relationship between handling treatments and brood size.

a)



b)

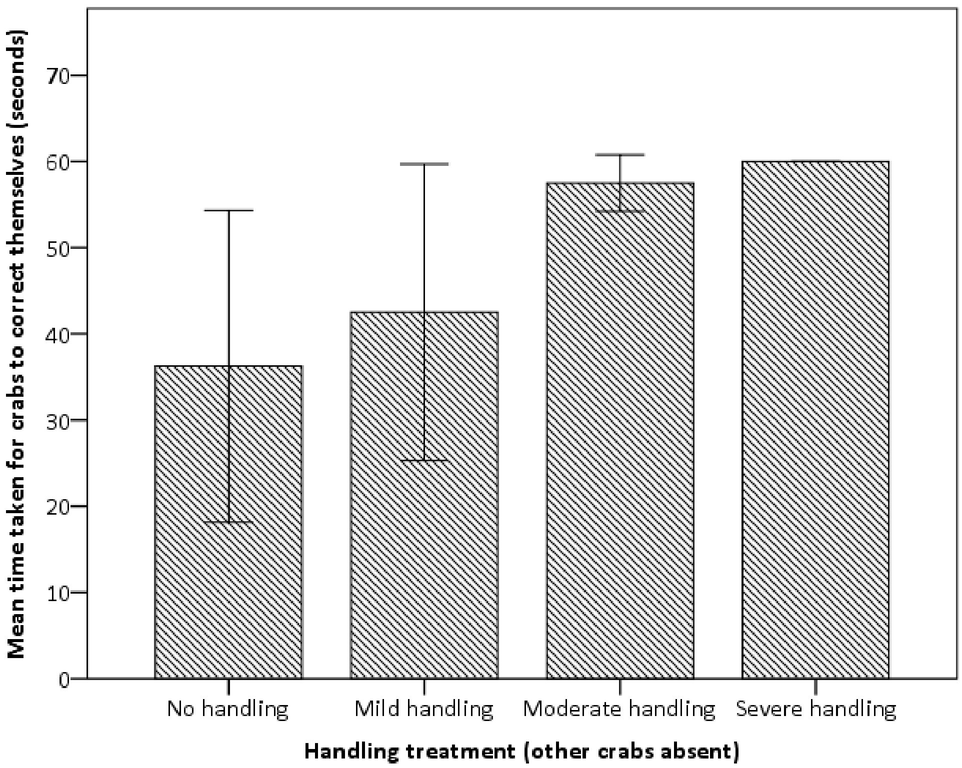


Figure 12. Mean (± S.E.) time taken for berried crabs to correct themselves (seconds) after handling treatment where other crabs were: a) present; and b) absent.

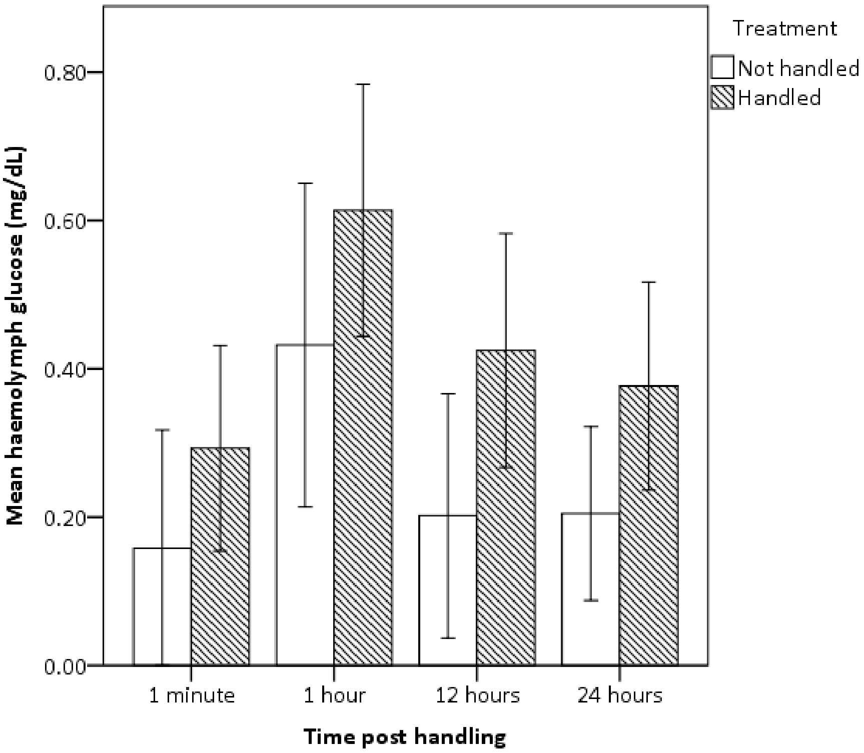
Experiment 3: Physiological responses of berried females to handling stress

Berried crabs (sample size = 40, carapace width range = 52 – 82 mm) were handled for one minute, and haemolymph samples taken immediately, 1 hour, 12 hours and 24 hours after handling to monitor the change in glucose and lactate levels using assay kits as above. In-between samples, crabs were kept in individual 3.5 litre plastic tanks (20cm length x 12cm width x 15cm height) with seawater flow through.

Results

Handling did not have a significant overall effect on physiological responses in berried velvet crabs. Glucose levels were similar between the handled and non-handled crabs throughout the experimental period (Fig. 13a). Levels of lactate in the haemolymph significantly increased 1 minute and 1 hour after handling; however after 12 hours, lactate returned to normal, in that levels were similar to those crabs not handled (Fig. 13b).

a)



b)

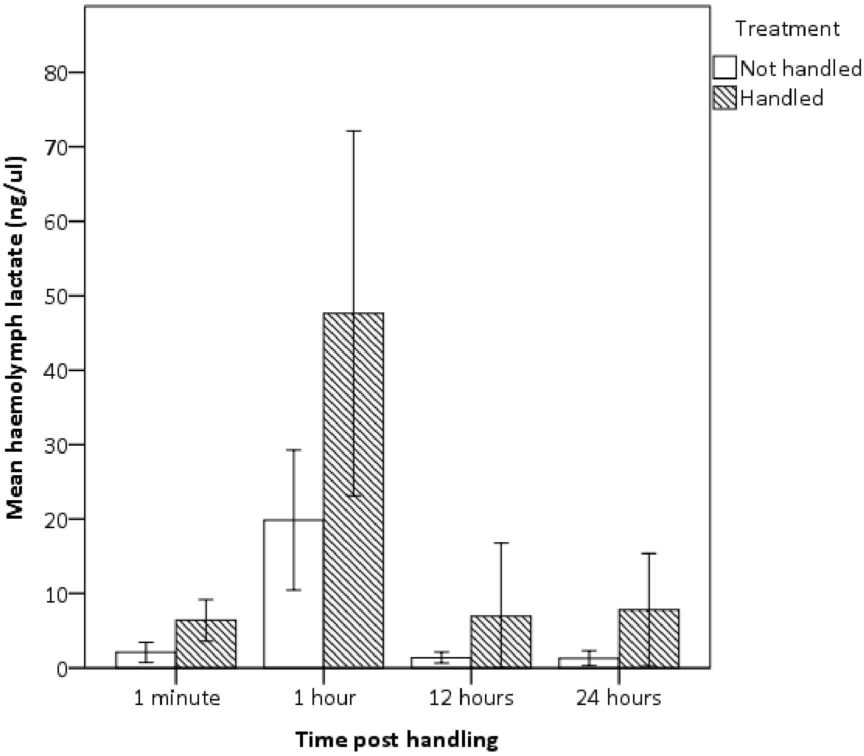


Figure 13. Mean (± S.E.) concentrations of: a) haemolymph glucose (mg/dL); and b) haemolymph lactate (ng/ul) in berried crabs after handling treatment

B3.3. Discussion

Experiment 1: Physiological effect of air exposure stress on velvet crabs

Short-term hyper-glycaemic responses can often be measured in crustaceans due to changes mediated rapidly by CHH (Keller and Andrew 1973, Patterson *et al.* 2007); here, no initial physiological changes were observed in haemolymph glucose of crabs after exposure to air exposure treatment for 10 minutes. However, as lactate is the main end product of anaerobic metabolism, it is also a good stress response indicator in crustaceans (Patterson *et al.* 2007). In this experiment, haemolymph lactate was significantly higher in treatment groups compared to the control after 10 minutes, indicating an initial stress response to air exposure. However, after 4 hours haemolymph lactate levels levelled out across the three groups, indicating similar stress levels.

Experiment 2: Behavioural assessment of handling on berried females

Severe handling resulted in berried crabs taking longer to correct themselves and return to their most natural position. This suggests that the stress associated with handling leaves berried crabs unable to maintain their upright, defensive position. Crabs generally took longer to turn over when other crabs were absent; however, crabs are tightly packed together during capture and transport and thus results from when other crabs are present most accurately reflects fishery practice and real life behaviours of berried crabs during capture. Handling did not have an effect on brood size.

Experiment 3: Physiological responses of berried females to handling stress

Handling appears to also cause a short term physiological response in berried crabs, as lactate levels in the haemolymph were significantly higher for at least an hour after handling (Fig. 13b). No difference between handling groups was detectable using glucose levels, indicating that handling does not cause a significantly high stress response in berried crabs (Fig. 13a).

B3.4. Conclusions

- **Crabs subjected to any sort of air exposure (whether or not they were covered in seaweed) exhibited a similar stress response. Therefore, where possible, they should kept in flowing seawater after capture.**
- **Berried females caught in creels but handled gently exhibit the same behavioural response as crabs that were not handled at all and crabs handled severely take longer to correct themselves to their natural position; therefore when returning berried crabs to the sea, they should be handled gently to increase survival.**
- **Handling did not have a negative impact on brood size in velvet crabs.**

B4. *Direct determination of age in* Necora puber *– pilot study*

B4.1. Project background

There are various methods by which age in aquatic animals can be determined, such as the otoliths in fishes (Campana 2001) and the shells of bivalves (Richardson *et al.* 2004). However, until recently no comparable structure has been found in crustaceans as they routinely shed their exoskeleton (referred to as 'moulting'). Because of this natural process, previous estimates of age in crustaceans was largely based on the length or size of the individual, with maturity also based on size rather than age. This is a problem, as some populations may have large individuals that are actually young and not reproductive, whereas other populations may have smaller individuals that are actually older and also reproducing. Setting a common minimum landing size for both populations could mean the first is fished unsustainably, whereas the second is under-utilised.

Research published in 2012 reported a new method for direct determination of age in crustaceans, including the snow crab (Kilada *et al.* 2012). This method is based on counting the growth bands deposited in the eyestalk, with strong evidence that the bands form irrespective of moult cycle and therefore is a reliable method of age determination. With some procedural adaptations, this method may be used to determine age in velvet crabs. This element of the project was a pilot of this method in velvet crabs and successfully developed several steps in the process.

B4.2. Methods

Crabs were chilled at -20^{o}C for three hours, then dissected and eyestalks removed. Procedures were carried out as follows for embedding in paraffin and resin:

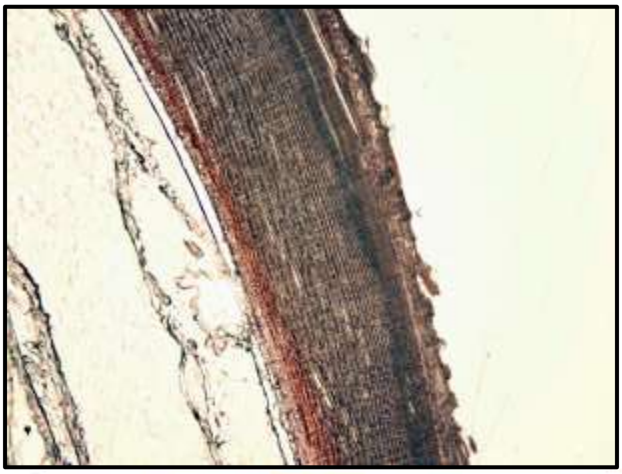
1. *Paraffin*: Eyestalks were decalcified in EDTA. Eyestalks were deemed to have decalcified when a needle point was able to be inserted into the eyestalk without force. Serial dehydrations were carried out in triplicate, each for half an hour (30%, 50%, 70%, 90%, 100%). Three 1:1 histoclear changes, each for half an hour were then carried out, followed by two changes in 100% histoclear. Eyestalks were embedded in paraffin and sections taken at 7µm.

2. *Resin*: Eyestalks were decalcified in formic acid, and deemed decalcified as above. Serial dehydrations were carried out in acetone, each for half an hour (30%, 50%, 70%, 90%, 100%). Eyestalks were embedded in resin and sections taken at 1.5µm.

B4.3. Results

Figure 14a shows a sample section taken through part of the cuticle surrounding the eye (paraffin section). Different structures are visible, with potential growth rings appearing as the differently coloured major bands (thinner red area, wider black area). In other longer-lived species multiple bands are visible, and the fine structure of cuticle is near visible inside the annual growth bands (Fig. 14b).

a)



b)

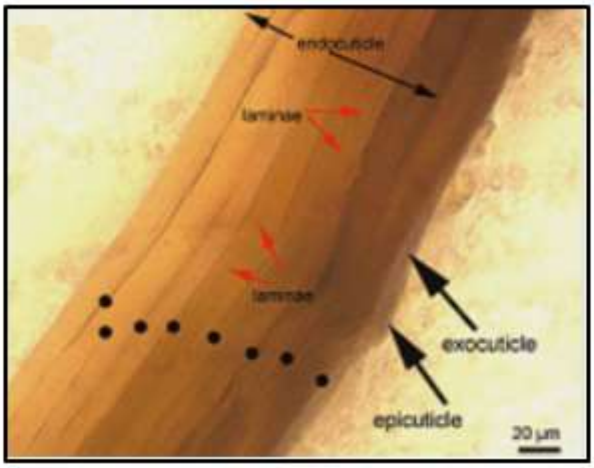


Figure 14. a) Section through velvet crab eye cuticle (1.5µm thick), showing fine structure of the cuticle (within a single annual growth band) (x40 magnification); and b) Reproduced from Kilada *et al.* (2012), showing section through eyestalk of the snow crab (*Chionoecetes opilio*) and multiple annual growth rings.

B4.4. Discussion

We were able to successfully remove, decalcify and dehydrate eyestalks, embed them in resin and paraffin, and produce 1.5-7 µm sections with our microtome. Structures were inspected under a microscope and potential growth bands identified. Although some differentiation in structures is visible (Fig. 14a, own data), the distinct age bands described by Kilada *et al.* (2012) (Fig. 14b) are not as clear in velvet crabs. This may be because the individuals used in the successful trials were less than 3 years old, thus only 1-2 growth bands are visible. It is also possible that in a different area of the eyestalk, the growth bands may be more readily visible. This pilot study shows great promise for the methodology and could resolve important age/ size/ maturity/ reproduction relationships in crustacean fisheries, which is recommended here for future research and development.

Anecdotal remarks, as well as results from population surveys, suggest that velvet crabs are generally larger in Strangford Lough than the Irish Sea; age is a potential factor that may explain the variation in crab size between the two areas, and potentially enable growth rates and size at maturity in the Lough and Irish Sea to be determined.

Generally, female crustaceans produce more eggs as they grow larger (Hartnoll 1985). Further studies may be carried out here to determine how egg masses in berried females are related to age of the individual in the different sites. If older females have a larger egg mass, then recruitment to populations could be enhanced if these females are not landed; however, age at maximum egg mass may differ between the Lough and Irish Sea as there is the notable difference in size. Understanding geographic variation in reproductive biology is key to a thorough understanding of species-specific life history patterns (Stearns 1976) and hence management of fisheries.

B4.5. Conclusion

A new technique for direct determination of age in crustaceans (Kilada *et al.* 2012), based on counting the growth bands deposited in the eyestalk, showed potential for determining age in velvet crabs. Further work should be carried out to fine-tune this technique.

B5. Summary of findings and recommendations

The two populations of velvet crabs studied here, inside Strangford Lough and in the adjacent Irish Sea, differed in body size and mass, and the peak timings of reproduction, that is, the proportions of females berried. We thus recommend closed seasons for berried crabs in the period December to April, but the exact period should be specifically tailored for each population alternatively a complete ban on landing berried crabs. Also, as body size is an unreliable measure of age and maturity, this means that minimum landing sizes should also be tailored for each population, following resolution of age/size relationships (eg with eyestalk growth band analyses).

There is an industry proposal to voluntarily increase the minimum landing size (MLS) for velvet crabs. This would serve to enhance populations by potentially allowing crabs a further opportunity to reproduce prior to landing and allow larger crabs, which have been shown to produce larger broods, to further enhance breeding and recruitment. To ensure that fishermen are not economically disadvantaged, a buy-back scheme for crab between the current legal MLS and the proposed higher voluntary MLS might be developed. This requires a reliable way to mark individual crabs to ensure that such crabs, when returned to Strangford Lough, are not landed before their next moult. The use of external markers as an indicator tool are not widely used in crustacean species due to moulting. Here, after trialling aerosol paint, Milliput and marine sealant silicone, the latter proved effective as an external marker for velvet crabs. The tag material itself did not elicit avoidance behaviour and, when applied to crabs, did not negatively influence crab behaviour with respect to feeding, mate choice or aggression. We thus recommend marine sealant silicone as the best substance with which to mark velvet crabs for potential future fisheries management.

Covering boxes of velvet crabs with damp seaweed does not appear to be sufficient to mitigate the stress effects of air exposure in the velvet crab. Some fishermen already attempt to minimise air exposure stress to the crabs by pumping seawater over the crabs while travelling between pots; however, the containers used to hold crabs allow water to escape and thus crabs are not submerged. Where possible, it is recommended that handling is minimised and crabs are contained within a flow-through seawater system while on board fishing vessels.

Behavioural assessments in berried crabs indicate that stress caused by harsh handling results in crabs taking longer to exhibit normal behaviour, but crabs handled gently showed the same behavioural response to crabs that were not handled. Berried crabs also exhibited a short term physiological response to handling. From this, we reccommend that crabs, particularly berried, are handled gently to mitigate the effects of handling stress. A ban on the landing of berried crabs would reduce the amount of handling that these vulnerable crabs are exposed to, with the added benefit of enhancing recruitment to the population.

SECTION C: Training and support

C1*. Summary of meetings, training and conferences*

Project steering group meetings

- 19th October 2012 (Seafish launch)
- 23rd October 2012
- 3rd December2013
- 20th February 2013
- 24th May 2013
- 27th September 2013
- 6th February 2014
- 29th October 2014
- 3rd February 2015
- 2nd July 2015

Training completed

- Powerboat handling course (RYA PB2 and Intermediate)
- Sea survival training (STCW78)
- Fire safety training
- Refworks for science
- Demonstrator at QUB training
- Lab techniques – microtome, staining procedures, assay kits

Conferences attended

- May 2013: Poster presentation at 10th Annual PG Marine Biological Association (Aberystwth, Wales)
- August 2013: Poster presentation at 28th Annual European Marine Biological Symposium (Galway, Rep. of Ireland)
- May 2014: Oral presentation at 11th Annual PG Marine Biological Association Conference (Scarborough, England)
- June 2014: Oral presentation at Seafish Northern Ireland Advisory Committee (Newcastle, N. Ireland)
- March 2015: Oral presentation at 107th Annual Meeting National Shellfisheries Association (Monterey, USA)
- May 2015: Oral presentation at 12th Annual PG Marine Biological Association Conference (Belfast, N. Ireland) (co-organiser)

Supervisory roles

- Undergraduate student honours project (2012-13)
- Undergraduate summer student (Summer 2013)
- A-level student (January 2014)
- Undergraduate summer student x2 (Summer 2014)
- Postgraduate student research project (Summer 2015)

C2. *Acknowledgements*

Many thanks are owed to Seafish for funding of this research, and to Prof. Dick, Dr. Sigwart and Dr. Gilmore for supervision and support. We would like to acknowledge and thank the support of fishermen for allowing access to their fishing vessels for population sampling, and for donating their personal time to attend steering group meetings. Steering group members provided valuable input: particularly Dick James (NIFPO) and Dr. Carrie McMinn (AFBI).

SECTION D: Supporting information

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D2. *Appendices*

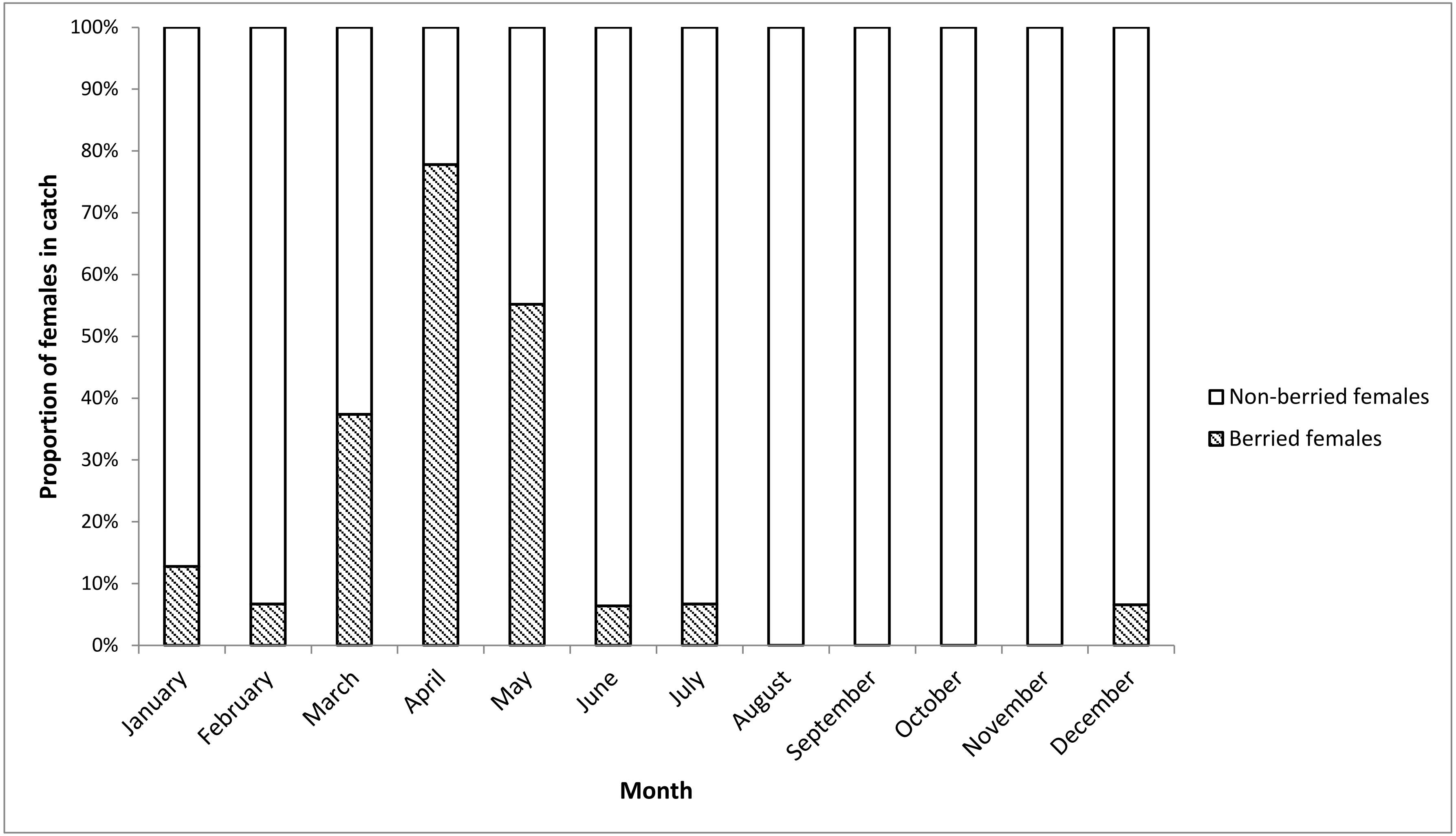
Appendix 1 - Site data logsheet used for population sampling

SKIPPER			**DATE**	
LOCATION			**STRING #**	
SITE DATA				
DEPTH	STRING #	POT #	# CRABS	OTHER SP.

Appendix 2 - Catch data logsheet used for population sampling

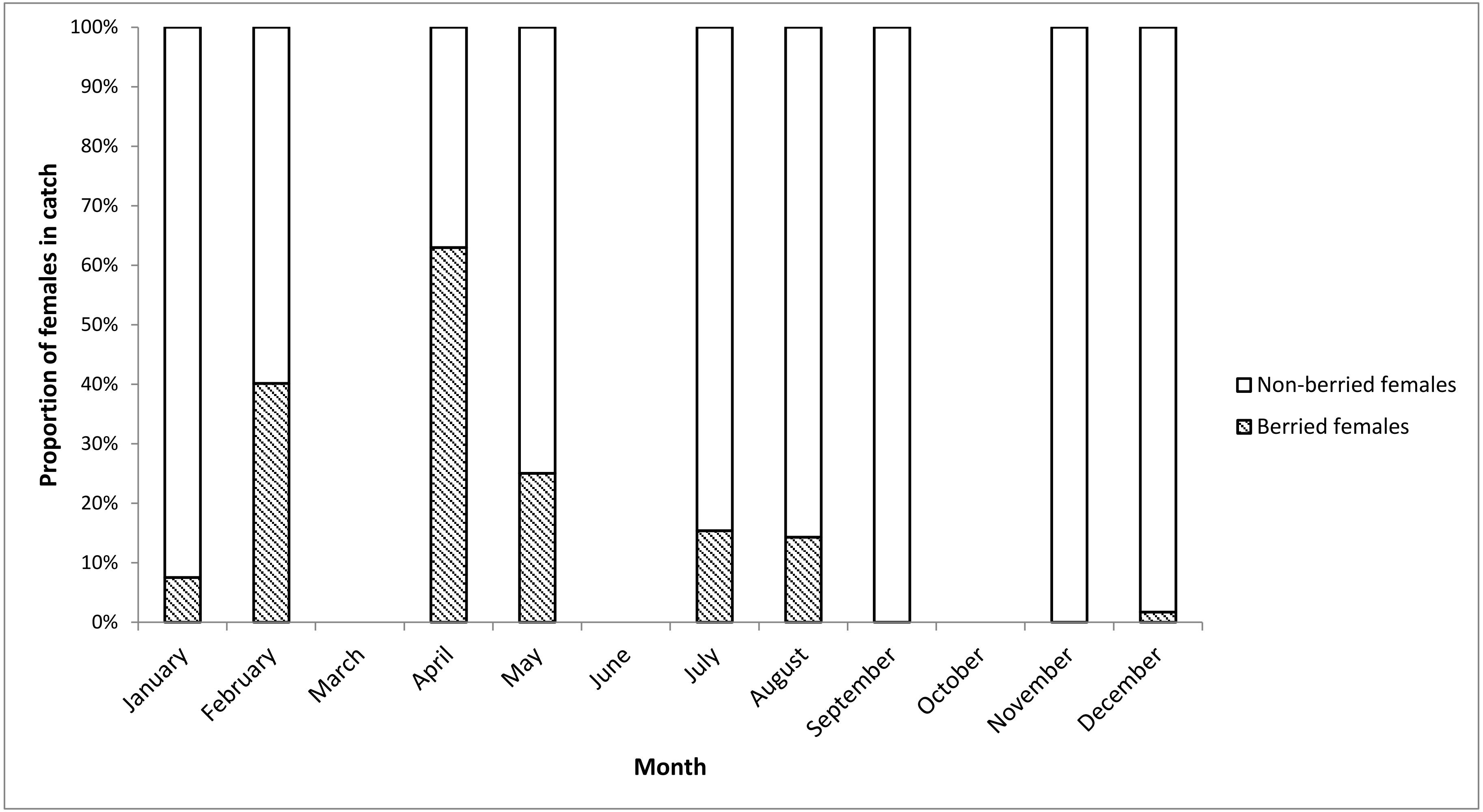
SKIPPER					**DATE**		
LOCATION					**STRING #**		
CATCH DATA							
SEX	MASS	CW	LIMB LOSS	MOULT	DISEASE	DAMAGE	BERRIED
M/F			**R**1 2 3 4 5 **L**1 2 3 4 5	0 1			
M/F			**R**1 2 3 4 5 **L**1 2 3 4 5	0 1			
M/F			**R**1 2 3 4 5 **L**1 2 3 4 5	0 1			
M/F			**R**1 2 3 4 5 **L**1 2 3 4 5	0 1			
M/F			**R**1 2 3 4 5 **L**1 2 3 4 5	0 1			
M/F			**R**1 2 3 4 5 **L**1 2 3 4 5	0 1			
M/F			**R**1 2 3 4 5 **L**1 2 3 4 5	0 1			
M/F			**R**1 2 3 4 5 **L**1 2 3 4 5	0 1			
M/F			**R**1 2 3 4 5 **L**1 2 3 4 5	0 1			
M/F			**R**1 2 3 4 5 **L**1 2 3 4 5	0 1			
M/F			**R**1 2 3 4 5 **L**1 2 3 4 5	0 1			
M/F			**R**1 2 3 4 5 **L**1 2 3 4 5	0 1			
M/F			**R**1 2 3 4 5 **L**1 2 3 4 5	0 1			
M/F			**R**1 2 3 4 5 **L**1 2 3 4 5	0 1			
M/F			**R**1 2 3 4 5 **L**1 2 3 4 5	0 1			
M/F			**R**1 2 3 4 5 **L**1 2 3 4 5	0 1			
M/F			**R**1 2 3 4 5 **L**1 2 3 4 5	0 1			
M/F			**R**1 2 3 4 5 **L**1 2 3 4 5	0 1			
M/F			**R**1 2 3 4 5 **L**1 2 3 4 5	0 1			
M/F			**R**1 2 3 4 5 **L**1 2 3 4 5	0 1			
M/F			**R**1 2 3 4 5 **L**1 2 3 4 5	0 1			
M/F			**R**1 2 3 4 5 **L**1 2 3 4 5	0 1			
M/F			**R**1 2 3 4 5 **L**1 2 3 4 5	0 1			
M/F			**R**1 2 3 4 5 **L**1 2 3 4 5	0 1			
M/F			**R**1 2 3 4 5 **L**1 2 3 4 5	0 1			

a)



100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Proportion of females in catch
January
February
March
April
May
June
July
August
September
October
November
December
Month
Non-berried females
Berried females

b)



Appendix 3 – Proportion of monthly berried and non-berried females in catch in: a) Strangford Lough; and b) Irish Sea (some months are omitted where no data is available)