



**Seasonal nutritional status in Norway lobsters, *Nephrops norvegicus* (L.): Are females nutritionally compromised over the winter?**

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1 **Seasonal nutritional status in Norway lobsters, *Nephrops norvegicus* (L.): Are females**  
2 **nutritionally compromised over the winter?**

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18  
19 **Running head**

20 Seasonal nutritional status in lobster *N. norvegicus*

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25

26 **Abstract**

27 Norway lobsters, *Nephrops norvegicus*, are sediment-dwelling decapod crustaceans that  
28 excavate burrows from which they make short excursions to feed by predation and  
29 scavenging. The females of this species are known to reside within their burrows for an  
30 extended period of time over the winter while brooding their eggs. The aim of this study was  
31 to assess the likelihood of these females being able to feed during this brooding period.  
32 Biophysical and biochemical measurements that had previously been shown to change with  
33 starvation under laboratory conditions in male *N. norvegicus* were taken for female *N.*  
34 *norvegicus* under similar conditions. These measurements were also compared in both  
35 sexes obtained from monthly trawl samples from the Clyde Sea Area, Scotland, UK, together  
36 with trawl composition data. The laboratory study showed that the hepatosomatic index, and  
37 the copper, lipid and water content of the hepatopancreas can be used as indicators of the  
38 state of starvation in females, as in males. In the wild, both sexes have reduced nutritional  
39 status during the winter, but not to the degree seen in animals starved for 20 weeks in  
40 aquarium trials. This study does not support the hypothesis that females cease feeding over  
41 winter, during their brooding period. Firstly, some females were unable to sustain ovary  
42 development during starvation under controlled conditions, contrary to field observations.  
43 Secondly, field data suggests that there is no sex-specific reduction in nutritional status.

44

45 **Key words**

46 *Nephrops norvegicus*, Norway lobster, nutritional state, starvation, brooding

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49 **Introduction**

50 Norway lobsters, *Nephrops norvegicus* (Linnaeus, 1758), are carnivores that emerge from  
51 their burrows to feed. Periods of burrow emergence vary in relation to ambient light  
52 conditions, sex and season (Bell et al. 2006). The nutritional status of a wild animal depends  
53 on a variety of factors, such as abundance and quality of food, search and capture ability,  
54 conspecific and interspecific competition, predator avoidance and periodical physiological  
55 changes (Macleod et al. 2008) Animals have developed coping mechanisms to deal with  
56 periods of food shortage, which range from reducing their metabolic rate to varying the  
57 extent to which tissue reserves are utilised to obtain energy (Roots 2006). Norway lobsters  
58 experience a number of these limiting nutritional conditions throughout their life.

59 Norway lobsters have been shown to be opportunistic predators and scavengers (Thomas &  
60 Davidson 1962) with stomach contents reflecting prey abundance rather than feeding  
61 preference (Bell et al. 2006). Fluctuations in primary production will influence the abundance  
62 of the benthic organisms on which they feed and may result in a reduction in food availability  
63 in the winter (Stephens et al. 1967). The nutritional status of a population of *N. norvegicus* in  
64 a particular area is also driven by density-dependent factors. In high density areas  
65 competition for food may limit scope for growth. Moreover, increased aggressive social  
66 behaviour in high density areas could drive up the metabolic rate and thus energy  
67 requirements (Chapman & Bailey 1987; Tuck et al. 1997b; Parslow-Williams 1998; Tuck et  
68 al. 1999; Bell et al. 2006; Campbell et al. 2009).

69 The reproductive cycle of female *N. norvegicus* shows latitudinal differences in the times of  
70 spawning, incubation, egg hatching and mating (Bell et al. 2006). Those in the Clyde Sea  
71 Area contain both annual and biennial spawners (Bailey 1984). Females become  
72 reproductively mature at around 3 years of age (Bell et al. 2006). As age is not easy to  
73 determine, due to the fact that there are no morphological structures that change in an age-  
74 related manner and are retained across successive moults, the size of the female is most  
75 often used to determine the stage at which it becomes reproductively active. This is known  
76 as the 'Size at Onset Maturity' or SOM, which has been defined by Bailey (1984) and Tuck  
77 et al. (1997a) as the size at which 50% of females ( $L_{50}$ ) have ovaries in a reproductively-  
78 active condition - an indicator of 'physiological maturity'. As an alternative indicator of SOM,  
79 both Bailey (1984) and Tuck et al. (1997a) have also used the size of the smallest ovigerous  
80 female as an indicator of 'functional maturity' and found no significant difference between  
81 these two methods.

82 Farmer (1974), Rotllant et al. (2005) and Mente et al. (2009) described in detail the  
83 development of the ovary maturation cycle. Immature females and those between

84 reproductive events have cream coloured ovaries. As they develop, the ovaries are coloured  
85 by a green vitellogen protein (Avarre et al. 2003). Ovary development commences during  
86 the winter, only reaching pale green coincident with emergence in spring. Most development  
87 takes place as a result of active feeding after spawning, moulting and mating (Farmer 1974).  
88 They mature through the spring and summer when animals are actively feeding, with egg  
89 laying occurring in late summer and autumn, after which females retreat to their burrows  
90 (Bell et al. 2006).

91 *Nephrops norvegicus* need to balance their food-searching behaviour with the need to avoid  
92 predators. These trade-offs are resolved by the animals staying in close proximity to their  
93 burrows during feeding excursions (Chapman & Rice 1971). Many of these limiting factors  
94 could be experienced by both sexes of *N. norvegicus*, but some will particularly affect  
95 ovigerous (so-called 'berried') females as they are known to reside within their burrows over  
96 winter while brooding their eggs, presumably in order to reduce their predation risk (Aguzzi  
97 et al. 2007). Newland (1985) showed that abdominal tail flipping (the method of escape  
98 swimming) is suppressed in egg-bearing *N. norvegicus* and that any flexions performed  
99 involve only the tail fan (uropods and telson) which does not carry eggs. Therefore there  
100 would be a greater predation risk for females in leaving their burrows to feed. Aguzzi et al.  
101 (2007) suggested that females can be attracted out of their burrows when food is available,  
102 as indicated by catching ovigerous females in creels, but then stay within close proximity to  
103 a burrow opening. With this limited foraging range, it is entirely possible that females reduce  
104 their feeding rate, or completely cease feeding. Aguzzi et al. (2007) showed that the  
105 percentage of ovigerous females with empty stomachs was significantly higher (60%) than  
106 that of non-ovigerous females (50%). Also, Oakley (1978) showed that ovigerous females  
107 were less likely to react to chemical food stimuli than either males or non-ovigerous females.  
108 Female *N. norvegicus* possibly enter a period of torpor while incubating their eggs in  
109 burrows, although there remains a requirement for them to ventilate and clean the eggs  
110 (Waddy et al. 1995). Such studies hypothesised that ovigerous females have a reduced  
111 nutritional state. It has been suggested that suspension feeding may provide an additional  
112 food source (Loo et al. 1993; Bell et al. 2006) but this is unlikely to be of benefit during the  
113 winter, though suspended matter derived from phytoplankton blooms may be available in  
114 late autumn and early spring, near the beginning and end of the period of burrow residence.

115 We have recently established that male *N. norvegicus* can survive for over 6 months without  
116 feeding (Watts et al. 2014), and since this is a similar length of time to the burrow residence  
117 time for female *N. norvegicus* it is entirely possible that they can also survive for this  
118 duration without feeding.

Comment [a1]: Comment1: addition

Comment [a2]: Comment2: deletion

119 Determining biomarkers of starvation that could be used reliably with specimens sampled in  
120 the field would help to show whether the females have a reduced nutritional status through  
121 the winter. Females are inaccessible for such assessments while they remain within their  
122 burrows (they are difficult to sample), but due to their relatively synchronised emergence  
123 from burrows post-brooding (Milligan et al. 2009), meaningful data can be obtained by  
124 targeting newly-emerged females.

125 Watts et al. (2014) identified appropriate measures for establishing the nutritional status of  
126 male *N. norvegicus* when they had not fed for 20 weeks. These were, the hepatosomatic  
127 index (HSI) and the water, lipid and copper content of the hepatopancreas and the carbon:  
128 nitrogen ratio of the abdominal tail muscle.

129 A reduction of whole body weight due to a reduced nutritional status does not occur within *N.*  
130 *norvegicus* since when reserves such as lipids become depleted in the hepatopancreatic  
131 tissue there is a corresponding increase in water content (Comoglio et al. 2005). In Male *N.*  
132 *norvegicus* lipid content decreased from an initial percentage of  $15.22 \pm 2.78$  to  $2.58 \pm 1.32$   
133 (wet weight), with a corresponding increase of water content from  $68.05 \pm 2.22\%$  to  $75.62 \pm$   
134  $2.39\%$  (Watts et al 2014). This was also seen by Karapanagiotidis et al. (2015) where crude  
135 lipids from the hepatopancreas (measured as dry weight) were  $50.22 \pm 1.28 \%$  in well fed *N.*  
136 *norvegicus* and  $13.35 \pm 1.19\%$  in *N. norvegicus* starved over similar time scales as Watts et  
137 al (2014). As a result, the combined weight of lipid and water held within the hepatopancreas  
138 did not significantly vary over time, remaining at approximately 80% of the tissue weight. The  
139 hepatopancreas itself however has also been shown to decrease in size with starvation.  
140 Therefore HSI (the proportion compared to whole body weight) can be an effective indicator  
141 of starvation in male *N. norvegicus*. Copper content of the hepatopancreas was shown in  
142 Watts et al. (2014) to increase with the level of starvation, from an initial  $153.53 \pm 42.17 \mu\text{g}$   
143  $\text{g}^{-1}$  to  $423.37 \pm 158.88 \mu\text{g g}^{-1}$  by week 12. This was also noted by Baden et al. (1994) with  
144 a drop in copper concentration in the haemolymph being accompanied by an increase in the  
145 hepatopancreas. Starvation showed very few effects on the constituents of the tail muscle:  
146 its water content did not vary significantly, perhaps due to the fact that it contains only ca.  
147 2% lipid. A decrease in lipid content was not directly recorded, although the carbon:nitrogen  
148 ratio, which is known to be a proxy indicator of lipid metabolism did decrease.

149 The aims of the present study were therefore to establish whether these measures of  
150 starvation effects in males were also appropriate for assessing the nutritional status of  
151 female *N. norvegicus* by performing a laboratory fasting experiment, and if so, to use such  
152 measures on newly emerged field-caught females to determine if they are in a reduced  
153 nutritional state (compared to males) having been confined to their burrows through the  
154 winter.

Comment [a3]: Comment3: addition

**155 Material and Methods**

156 The study comprised an aquarium investigation of the effect of food deprivation on the  
157 biophysical dimensions and biochemical reserves of female *N. norvegicus* over a period of  
158 approximately 6 months. Biophysical and biochemical measurements were taken from field-  
159 caught individuals, and then compared with those from the aquarium study to assess the  
160 likelihood of food deprivation in the wild and thus the nutritional status for both males and  
161 females. Data presented in Watts et al. (2014) were used to compare the nutritional status of  
162 males in the field.

**163 Aquarium study: physiological effects of long-term starvation**

164 *Nephrops norvegicus* were collected (Oct 2010) by trawling from the Clyde Sea Area (CSA),  
165 Scotland, UK along an established transect north of the Isle of Cumbrae (55°51.35'N  
166 4°54.42'W to 55°48.97'N 4°54.05'W) (Beevers et al. 2012; Watts et al. 2014). Females with a  
167 mean ( $\pm$  SD) carapace length of 33.2 mm  $\pm$  5.1 mm were selected randomly (n=62) and  
168 transferred to a recirculating natural sea water aquarium (12h:12h light: dark photoperiod, at  
169 9.4 °C  $\pm$  0.6 °C SD) in the University of Glasgow for two weeks. All animals were fed with ca  
170 1g squid mantle three times a week (the 'standard food ration') for an initial 2-week period,  
171 until the trial started on 26 October 2010.

172 Animals were assigned randomly to one of 14 numbered tanks. The animals in two tanks  
173 had the standard food ration ('fed' group), and food was removed from the tanks after 20 h.  
174 Those in the other ~~ten~~ 12 tanks were starved ('unfed' group). One unfed animal was  
175 removed from each even-numbered tank at weeks 0, 8 and 16, and one unfed animal was  
176 removed from each odd-numbered tank at weeks 4, 12 and 20. As there were no major  
177 changes in nutritional status over the course of the trial in the male fed group (Watts et al.  
178 2014), the fed group of females was sampled once at week 20.

179 Animals were put on ice for 20 minutes prior to measurements of carapace length (with dial  
180 callipers) and weight without claws (to avoid differences due to claw loss during trawling and  
181 post-capture handling). The hepatopancreas and ovary were then removed from the  
182 cephalothorax (noting the colour and weight of the organs), and all muscle tissue was  
183 removed from the abdomen. These three tissues were frozen in liquid nitrogen and then  
184 stored at  $-80^{\circ}\text{C}$  prior to further analysis.

185 The biophysical measure of hepatosomatic index and the biochemical measures of  
186 hepatopancreas water, lipid and copper content, along with abdominal tail muscle protein,  
187 water, the stable isotopes of  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and the carbon-nitrogen ratio (C:N), were made on  
188 each animal, using methods described by Watts et al. (2014).

Comment [a4]: Comment4: correction

189 *Field study*

190 Starting in January 2009, *Nephrops norvegicus* were collected monthly (January-December)  
191 by trawling along the established transect in the CSA mentioned above. Three research  
192 vessels from University Marine Biological Station Millport were used throughout the sampling  
193 period (RV Aora, RV Aplysia and RV Actinia), each equipped with an otter trawl with a mesh  
194 size of 70 mm at the cod-end. Trawls lasted for 1 h, at a mean depth of  $78.5 \text{ m} \pm 1.1 \text{ m SD}$ .

195 *Assessment of reproductive state*

196 A sub-sample of *Nephrops norvegicus* was taken non-selectively from each catch before any  
197 other animals were removed for other purposes. A random sample of the catch was placed  
198 into a fishing basket. The bycatch was then removed and discarded, leaving approximately  
199 200 *N. norvegicus* (depending on the size of the catch). When the catch had fewer than 200  
200 animals the entire catch was recorded. The sample was placed in a polystyrene box and  
201 covered with ice and transported to the University of Glasgow. This sample was measured  
202 within 24h of the catch, when not measured the same night the animals were refrigerated  
203 overnight ( $5^{\circ}\text{C}$ ).

204 The carapace length, weight without chelipeds, and sex of all *N. norvegicus* were recorded.  
205 The colour and weight of the hepatopancreas, and the colour and weight of the ovaries in  
206 females, were also recorded in a randomly selected sample of 25 of each sex.

207 *Assessment of nutritional state*

208 An additional 15 *Nephrops norvegicus* of each sex were sampled from the remainder of the  
209 catch from each haul. Their moult stage, according to carapace hardness, was determined  
210 as pre/post moult (soft), immediate post-moult ('jelly') or intermoult (hard) (Milligan et al.  
211 2009). Intermoult animals were taken preferentially, and postmoult animals were used only  
212 when low numbers of animals were caught. Animals were put on ice and taken ashore were  
213 processed within 4h. The carapace length, body weight, hepatopancreas colour and weight,  
214 and gonad colour, stage and weight were recorded. Samples of abdominal tail muscle and  
215 hepatopancreas were taken, frozen in liquid nitrogen and transported to the University of  
216 Glasgow where they were stored at  $-80^{\circ}\text{C}$  until all samples were collected and processed  
217 further. Biophysical and biochemical measures were then taken for each animal and  
218 assessed against the biophysical and biochemical measurements (see Section 2.1).

219 *Statistics*

220 Variation in biophysical or biochemical measurements over time and in relation to nutritional  
221 status were tested with general linear models (GLM, computed in the statistical program  
222 Minitab). For unfed animals in the aquarium study the factor 'time' was represented by



223 weeks since last fed as a categorical variable. Differences between fed and unfed animals  
224 were tested in *Nephrops norvegicus* sampled at week 20. For the field study the factor 'date'  
225 was represented by the months caught, with males and females assessed separately.  
226 Normality of residuals and homogeneity of variances were assessed visually. A significant  
227 result was determined when  $p < 0.05$  and the Tukey multiple comparison procedure was  
228 followed for both studies.

229

## 230 Results

### 231 Aquarium study

232 The results obtained from the fed and unfed groups of female *Nephrops norvegicus* during  
233 the 20 week experimental period are presented in Figure 1 (means + SE). Values of fed and  
234 unfed females at week 20 are shown in Table SI (supplementary material).

235 The concentration of copper in the hepatopancreas in unfed females increased significantly  
236 between week 0 and week 20 ( $F_{5,35}=3.45$ ,  $p=0.014$ ) (Figure 1A). There was also a significant  
237 difference in the copper concentration in the hepatopancreas between fed and unfed  
238 animals at week 20 ( $F_{1,10}=10.25$ ,  $p=0.011$ ).

239 The HSI in unfed females decreased significantly between week 0 and week 4 and then  
240 remained relatively constant throughout the rest of the trial ( $F_{5,35}=5.60$ ,  $p=0.001$ ) (Figure 1B).  
241 At week 20, fed females had a significantly higher HSI than unfed animals ( $F_{1,10}=12.61$ ,  
242  $p=0.006$ ).

243 The lipid concentration in the hepatopancreas in unfed females initially increased between  
244 week 0 and weeks 8–16 and then decreased to lower than the initial value ( $F_{5,28}=4.89$ ,  
245  $p=0.003$ ) (Figure 1C). Hepatopancreas lipid content was significantly lower in unfed female  
246 *N. norvegicus* at week 20 ( $F_{1,9}=10.45$ ,  $p=0.012$ ).

247 The water content in the hepatopancreas in unfed females did not increase significantly  
248 between week 0 and week 20 ( $F_{5,35}=2.29$ ,  $p=0.071$ ) (Figure 1D). However, there was a  
249 significant difference in the water content of the hepatopancreas between fed and unfed  
250 female *N. norvegicus* at week 20, being higher in unfed females ( $F_{1,8}=10.13$ ,  $p=0.015$ ).

251 For female *N. norvegicus* there was no significant variation among weeks or between fed  
252 and unfed groups in the measurements of abdominal tail muscle water ( $F_{5,35}=0.86$ ,  $p=0.518$ ;  
253  $F_{1,8}=3.31$ ,  $p=0.112$ ), abdominal tail muscle  $\delta^{13}\text{C}$  ( $F_{5,35}=2.03$ ,  $p=0.103$ ;  $F_{1,8}=1.63$ ,  $p=0.243$ ),  
254 abdominal tail muscle  $\delta^{15}\text{N}$  ( $F_{5,35}=0.54$ ,  $p=0.745$ ;  $F_{1,8}=1.38$ ,  $p=0.279$ ) or the abdominal tail  
255 muscle C:N ratio ( $F_{5,35}=0.69$ ,  $p=0.635$ ;  $F_{1,8}=1.02$ ,  $p=0.347$ ) (Supplementary Material Table  
256 SI).

257 The proportion of each ovary stage at each time period is shown in Figure 2. Within the first  
 258 4 weeks four out of six females reabsorbed their ovaries. From the females that were fed  
 259 throughout the trial, two of them displayed white gonads and one had cream gonads,  
 260 showing no utilisation of ovaries as reserves in these individuals during the trial. Ovary  
 261 development did not occur within half of the unfed individuals, but rather there was an  
 262 increasing amount of reabsorption.

### 263 *Field studies*

#### 264 *Nutritional Status*

##### 265 *Females*

266 Only one female was collected in the whole catch in January, therefore this month was  
 267 removed from all further analyses. Only mature females (<26mm) were used for nutritional  
 268 status measurements. [Average sizes of male and females each month can be seen in](#)  
 269 [TableSII.](#)

270 When female *Nephrops norvegicus* were measured monthly throughout 2009 there was  
 271 significant seasonal variation in hepatopancreas copper concentration ( $F_{10,115}=3.98$ ,  
 272  $p<0.001$ ) ([Figure 3A](#)), [water content \( \$F\_{9,133}=9.90\$ ,  \$p<0.001\$ \)](#) and [lipid content \( \$F\_{9,67}=5.29\$ ,](#)  
 273  [\$p<0.001\$ \)](#) ([Figure 3E](#)), and [water content \( \$F\_{9,133}=9.90\$ ,  \$p<0.001\$ \)](#) ([Figure 3G](#)). However there  
 274 was no significant variation in HSI ( $F_{7,107}=1.76$ ,  $p=0.104$ ) ([Figure 3C](#)). Females caught in  
 275 February had an elevated copper concentration in the hepatopancreas, as found in males  
 276 but to a smaller extent. The water content of the hepatopancreas was high in the months  
 277 February–June and lipid content of the female hepatopancreas was 14–24% throughout the  
 278 year and only exceeded 30% ( $30.5 \pm 3.47\%$ ) in December. (Table [SIII4A](#)).

##### 279 *Males*

280 In males, there was significant seasonal variation in hepatopancreas copper concentration  
 281 ( $F_{10,129}=15.22$ ,  $p<0.001$ ) ([Figure 3B](#)), HSI ( $F_{7,112}=5.17$ ,  $p<0.001$ ) ([Figure 3D](#)), [hepatopancreas](#)  
 282 [lipid content \( \$F\_{9,63}=4.34\$ ,  \$p<0.001\$ \)](#) ([Figure 3F](#)) and [hepatopancreas water content](#)  
 283 [\( \$F\_{10,140}=7.72\$ ,  \$p<0.001\$ \)](#) ([Figure 3H](#)), and [hepatopancreas lipid content \( \$F\_{9,63}=4.34\$ ,  \$p<0.001\$ \)](#).

284 The hepatopancreas copper values in males collected from the field in February were  
 285  $420.34 \pm 69.81 \mu\text{g}\cdot\text{g}^{-1}$ . Males caught between February and April also had values of  
 286 hepatopancreas water and lipid content comparable to the values observed in unfed  
 287 individuals (Table [SIII-4B](#)). Later in the year (September) all parameters measured indicated  
 288 that males were well fed with values closer to the fed group (aquarium trial).

##### 289 *Female reproductive state*

290 The seasonal pattern in the proportion of all females caught in the trawl catches along the  
 291 sampled transect is shown in Figure [3A4A](#). The main features for females were low numbers

**Comment [a5]:** Comment 5: addition of figure references

**Comment [a6]:** Comment 5: addition of figure references

**Comment [a7]:** Comment 6: correction, along with two other occurrences below

292 during the winter months, an increasing proportion in the spring, peak abundance in the  
293 summer, a declining proportion in the autumn and a return to low numbers in the winter.

294 The seasonal pattern in the proportion of females in each stage of ovary maturation is shown  
295 in Figure 3B4B. The females caught in the winter months had less-well developed ovaries  
296 than those in the summer (although as shown in Figure 4A some of these may be  
297 immature). A progression in the maturation cycle can be seen within the first half of the year  
298 (January–July). In April 50% females had green ovaries, and were therefore mature and  
299 ready to mate. This increased to 87.8% in July as all females sampled had ovaries  
300 developed to stage 3 or beyond. In the second half of the year an increased proportion of  
301 females caught had spent ovaries, indicating that they had spawned recently.

Comment [a8]: Comment7: Addition to clarify

302 The seasonal pattern in the proportion of females with eggs is shown in Figure 3C4C.  
303 Ovigerous females were caught in highest numbers in September with 37% of all females  
304 being berried prior to their winter burrow period. Only 1% and 4% of the newly emerged  
305 females in April and May respectively were ovigerous suggesting that they have released the  
306 eggs prior to the collection.

307 Expressing the sizes of both sexes according to size categories (10–19.9, 20–29.9, 30–39.9,  
308 40–49.9, and 50+ mm CL) a distinct sex difference was found in the seasonal fluctuation of  
309 body size of the animals in the random sample (Figure 45A Females, Figure 3B-5B Males).

Comment [a9]: Comment 8: correction

310 The size distribution of the males fluctuated throughout the year. Females caught in the  
311 winter were mainly less than 30 mm CL, while in the summer there was a greater proportion  
312 in the 30–39.9 mm CL category. The dotted line indicates the division between mature  
313 (above) and immature (below) female *Nephrops norvegicus*. This shows that a higher  
314 proportion of female *N. norvegicus* caught in the months of February, November and  
315 December were immature.

316

## 317 Discussion

318 The aim of this study was to determine if the burrow-bound behaviour of female *Nephrops*  
319 *norvegicus* through the winter months, which is related to their annual reproductive cycle,  
320 leads to a reduced nutritional state compared to males.

321 To do this, biochemical and biophysical measurements that have been shown to indicate the  
322 nutritional state of male *N. norvegicus* (Watts et al. 2014) were determined in females both  
323 under controlled conditions and from the wild. In males, the HSI, hepatopancreas copper  
324 concentration, lipid and water content all changed significantly during starvation under  
325 controlled conditions (Watts et al., 2014). HSI and lipid content decreased as the

326 hepatopancreas decreased in size, whereas the water and copper content of the  
327 hepatopancreas increased due to a replacement of lipids with water. In the present study, in  
328 starved females (i.e. food deprivation) the HSI, hepatopancreas copper, lipid and water  
329 content also changed due to nutritional limitation (as in males). However the C:N ratio of  
330 female abdominal tail muscle did not decrease significantly in unfed individuals as it did in  
331 males. Also females maintained lipid reserves longer in the hepatopancreas (around 31%  $\pm$   
332 6.9% remaining at week 16, compared to 13% in males).

333 It is likely therefore that under nutritionally-limited conditions females will utilise lipids stored  
334 in the hepatopancreas only as a last resort. Females will still develop ovaries up to stage 2  
335 over the 6 months of the burrow dwelling period (Farmer 1974). Tuck et al. (1997c) suggest  
336 that starvation could be one reason for ovary reabsorption. In the present study, ovary  
337 development did not occur within half of the unfed individuals, but rather there was an  
338 increasing amount of reabsorption. Thus, for the population to remain stable females would  
339 still have to consume food to gain energy for this development. For reproductive events to  
340 take place in the following years it is likely that females will have to eat whilst in their  
341 burrows. The sex ratios recorded monthly through the year conformed to the traditional  
342 pattern of increasing emergence of females from their burrows during the spring (Oakley  
343 1978; Bailey 1984; Briggs 1995; Tuck et al. 1997a; Milligan et al. 2009). Over the winter,  
344 females were far less abundant in trawl catches, consistent with them spending much of  
345 their time within their burrows. Females in the summer dominated catches rising to 70% of  
346 all *N. norvegicus* in the catch; this was also seen in Milligan et al 2009 who sampled the  
347 same population in 2005-2006. The reason for this is currently unclear however could relate  
348 to an increased exploitation of males over the winter, or females having greater foraging  
349 dominance than males at this time. This would be interesting to investigate further.

350 Furthermore, females captured in trawls during the winter months were small (<30mm CL)  
351 and in December around 50% were immature. Thomas & Figueiredo (1965) showed that  
352 immature females show no seasonal variation in capture abundance. Females caught in  
353 April and May therefore include a high proportion of the females emerging from their burrows  
354 after egg hatching, and their nutritional status may thus reflect a long period of burrow  
355 residence. Consistent with Milligan et al. (2009) the number of females appearing in the May  
356 catches was higher than that of males (70% females, 30% males). It is not clear why this is  
357 not 50:50.

358 In field-caught *N. norvegicus* there was significant variation in lipid, water and copper content  
359 of the hepatopancreas measured in both female and male individuals throughout the year.  
360 The results obtained from animals caught from February to June indicated that during this  
361 period animals may have a reduced nutritional status compared with that seen in July to

**Comment [a10]:** Comment 9: addition of suggestion why Females dominant in the catch.

362 December. Copper concentrations in the hepatopancreas were high in February in both  
363 males and females. Lipids and water contents of the hepatopancreas were also elevated.  
364 These findings suggest that during the winter both male and female *N. norvegicus* have  
365 reduced nutritional status, but not to the degree seen after 20 weeks of starvation in the  
366 aquarium trials. Temperatures in the aquarium trials were constant with temperatures in the  
367 summer, but around 2°C higher than in the winter. Therefore the greater effect seen in the  
368 aquarium trial could be due to an increased metabolism in these animals. There is no  
369 evidence that females had a lower nutritional status than males when comparing sex specific  
370 starvation rates (even though females had higher copper values than males from May to  
371 October and lipids from May to September). In September, when the maximum numbers of  
372 ovigerous females were found, their nutritional state was consistent with that of fed animals  
373 in the aquarium trial. This suggests that females are entering their winter burrowing period in  
374 a good nutritional state. This is consistent with the results from quarterly samples of *N.*  
375 *norvegicus* taken in 1992–93 (IMBC et al. 1994) and bimonthly samples in 1995–96  
376 (Parslow-Williams 1998). Those studies showed that the lowest lipid and highest water in the  
377 hepatopancreas occurred in the spring and vice versa for the summer. However, HSI did not  
378 vary significantly between these two seasons in this study.

379 It is therefore likely that sex specific interactions play a fundamental part in the nutritional  
380 status of *N. norvegicus*. Reasons for the reduced nutritional status could include lower food  
381 availability, and less consumption of the food that is present. As explained in the  
382 introduction, food available to benthic organisms ultimately comes from the water column  
383 above, and in the winter months there is a reduction in primary production in the water  
384 column and thus the amount being driven down to the benthos. This theory was also  
385 suggested by IMBC et al. (1994). Less consumption of any food that is present is also  
386 possible during the winter due to a reduced burrow emergence. This might be as a response  
387 to lower light levels, or lower temperatures causing *N. norvegicus* to remain in their burrows  
388 for more extended periods of time in the winter months (Maynou & Sarda 2001). It is known  
389 that spring represents the time of post-brooding burrow emergence of females in the Clyde  
390 population of *N. norvegicus* (Milligan et al., 2009), and similarly in our study April was the  
391 first month in which the proportion of females in the catch reached 50%. At this time female  
392 *N. norvegicus* do seem somewhat limited nutritionally (due to a high hepatopancreas water  
393 percentage), but, when considering hepatopancreas copper concentrations, no more so than  
394 male *N. norvegicus* and less so than females in March and May. Oakley (1978) observed  
395 that in the winter months of a controlled experiment under artificial conditions (November–  
396 May) ovigerous females rarely came out beyond 25 cm from the burrow entrance to collect  
397 food that had been detected, and carried large food items into their burrow systems for

398 consumption. It is also known that *N. norvegicus* will bury food within their burrows, and  
399 females kept for an extended period of time in a mesocosm have shown 'caching' behaviour  
400 by burying food items away from the burrow, presumably so as not to attract predators  
401 (Atkinson & Eastman 2015). This suggests that females could in fact 'prepare' for the winter  
402 months by creating a readily accessible food store.

403 In conclusion, this study does not support the hypothesis that females cease feeding over  
404 winter. Firstly, some females were unable to sustain ovary development during starvation  
405 under controlled conditions, contrary to field observations. Secondly, field data suggest that  
406 there is no sex-specific reduction in nutritional status.

407

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412

413 **References**

- 414 Aguzzi J, Company JB, Sarda F. 2007. The activity rhythm of berried and unberried females  
415 of *Nephrops norvegicus* (Decapoda, Nephropidae). *Crustaceana* 80:1121-134.
- 416 Atkinson RJA, Eastman LB. 2015. Burrow dwelling in Crustacea. In: Thiel M, Watling L,  
417 editors. *Lifestyle and feeding biology. The Natural History of the Crustacea Vol 2*, Oxford:  
418 Oxford University Press, p 78-117.
- 419 Avarre JC, Michelis R, Tietz A, Lubzens E. 2003. Relationship between vitellogenin and  
420 vitellin in a marine shrimp (*Penaeus semisulcatus*) and molecular characterization of  
421 vitellogenin complementary DNAs. *Biology of Reproduction* 69:355-64.
- 422 Bailey N. 1984. Some aspects of reproduction in *Nephrops*. International Council for the  
423 Exploration of the Sea C.M. 1984/K:33:1-39.
- 424 Beever ND, Kilbride E, Atkinson RJA, Neil DM. 2012. A re-evaluation of *Hematodinium*  
425 infection seasonality in the Firth of Clyde Norway lobster (*Nephrops norvegicus*)  
426 population, Scotland. *Diseases of Aquatic Organisms* 100(2): 95-104.
- 427 Bell MC, Redant F, Tuck ID. 2006. *Nephrops* Species. In: Phillips BF, editor. *Lobster.*  
428 *biology, management, aquaculture and fisheries*. Oxford: Wiley-Blackwell Scientific  
429 Publishing, p 412-461.
- 430 Briggs RP. 1995. Variability in northwest Irish Sea *Nephrops* populations. *Fisheries*  
431 *Research* 23:175-87.
- 432 Campbell N, Allan L, Weetman A, Dobby H. 2009. Investigating the link between *Nephrops*  
433 *norvegicus* burrow density and sediment composition in Scottish waters. *ICES Journal of*  
434 *Marine Science* 66:2052-9.
- 435 Chapman CJ, Rice AL. 1971. Some direct observations on ecology and behaviour of Norway  
436 Lobster *Nephrops norvegicus*. *Marine Biology* 10:321-9.



- 437 Chapman CJ, Bailey N. 1987. Biological research on fish and shellfish stocks- Recent  
438 progress in Norway lobster research. In: Bailey RS, Parrish BB, editors. Developments in  
439 fisheries research in Scotland. Farnham: Fishing News Books, p 99-111.
- 440 Farmer ASD. 1974. Reproduction in *Nephrops norvegicus* (Decapoda: Nephropidae).  
441 Journal of Zoology 174:161-83.
- 442 IMBC, UMBSM, IRPEM. 1994. *Nephrops norvegicus*: stock variability and assessment in  
443 relation to fishing pressure and environmental factors. Final report to EC, Contract XIV-  
444 1/MED/91/003. Institute of Marine Biology of Crete, IMBC, University Marine Biological  
445 Station Millport, Istituto di Ricerche sulla Pesca Marittima, Crete. [84 pages](#).
- 446 Karapanagiotidis IT, Mente E, Berillis P, Rotllant G. 2015. Measurement of the feed  
447 consumption of feeding on different diets and its effect on body nutrient composition and  
448 digestive gland histology. Journal of Crustacean Biology 35:11-19.
- 449 Loo LO, Baden SP, Ulmestrand M. 1993. Suspension feeding in adult *Nephrops norvegicus*  
450 (L.) and *Homarus gammarus* (L.) (Decapoda). Netherlands Journal of Sea Research  
451 31:291-7.
- 452 Macleod R, Clark J, Cresswell W. 2008. The starvation-predation risk trade-off, body mass  
453 and population status in the Common Starling *Sturnus vulgaris*. Ibis 150:199-208.
- 454 Maynou F, Sarda F. 2001. Influence of environmental factors on commercial trawl catches of  
455 *Nephrops norvegicus* (L.). ICES Journal of Marine Science 58:1318-25.
- 456 Mente E, Karapanagiotidis IT, Logothetis P, Vafidis D, Malandrakis E, Neofitou N, et al.  
457 2009. The reproductive cycle of Norway lobster. Journal of Zoology 278:324-32.
- 458 Milligan RJ, Albalat A, Atkinson RJA, Neil DM. 2009. The effects of trawling on the physical  
459 condition of the Norway lobster *Nephrops norvegicus* in relation to seasonal cycles in the  
460 Clyde Sea Area. ICES Journal of Marine Science 66:488-94.
- 461 Newland PL. 1985. The control of escape behaviour in the Norway Lobster, *Nephrops*  
462 *norvegicus* (L). PhD Thesis, University of Glasgow, pp. 38-60. [158 pages](#).
- 463 Oakley S. 1978. Food, feeding behaviour and some aspects of the ecology of *Nephrops*  
464 *norvegicus* in the Irish Sea. PhD thesis, University of Liverpool. [231 pages](#).
- 465 Parslow-Williams PJ. 1998. Nutritional limitation in populations of the Norway lobster,  
466 *Nephrops norvegicus* (L.) in the Firth of Clyde, Scotland. PhD thesis. University of  
467 Glasgow. [238 pages](#)
- 468 Roots C. 2006. Hibernation. London: Greenwood Press, [219 pages](#).



- 469 Rotllant G, Ribes E, Company JB, Durfort M. 2005. The ovarian maturation cycle of the  
470 Norway lobster *Nephrops norvegicus* (Linnaeus, 1758) (Crustacea, Decapoda) from the  
471 western Mediterranean Sea. *Invertebrate Reproduction and Development* 48:161-9.
- 472 Stephens K, Sheldon RW, Parsons TR. 1967. Seasonal variations in availability of food for  
473 benthos in a coastal environment. *Ecology* 48:852-5.
- 474 Thomas HJ, Davidson C. 1962. The food of the Norway lobster. *Marine Research* 3:1-15.
- 475 Thomas HJ, Figueiredo MJ. 1965. Seasonal variations in the catch composition of the  
476 Norway lobster, *Nephrops norvegicus* (L.). *Journal du Conseil / Conseil Permanent*  
477 *International pour l'Exploration de la Mer* 30:75-85.
- 478 Tuck ID, Chapman CJ, Atkinson RJA. 1997 a. Population biology of the Norway lobster,  
479 *Nephrops norvegicus* (L) in the Firth of Clyde, Scotland - I: Growth and density. *ICES*  
480 *Journal of Marine Science* 54:125-35.
- 481 Tuck ID, Chapman CJ, Atkinson RJA, Bailey N, Smith RSM. 1997 b. A comparison of  
482 methods for stock assessment of the Norway lobster, *Nephrops norvegicus*, in the Firth of  
483 Clyde. *Fisheries Research* 32:89-100.
- 484 Tuck ID, Taylor AC, Atkinson RJA, Gramitto ME, Smith C. 1997 c. Biochemical composition  
485 of *Nephrops norvegicus*: changes associated with ovary maturation. *Marine Biology*  
486 129:505-11.
- 487 Tuck I, Bailey N, Atkinson J, Marrs S. 1999. Changes in *Nephrops* density in the Clyde Sea  
488 Area, from underwater TV survey data. Study group on life history of *Nephrops*. *ICES CM*  
489 1999/G:13, p 24-31.
- 490 Waddy SL, Aiken DE, de Kleijn DPV. 1995. Control of Growth and Reproduction. In: Factor  
491 JR, editor. *Biology of Lobsters Homarus americanus*. San Diego, California: Academic  
492 Press Inc., p 217-66.
- 493 Watts AJR, McGill RAR, Albalat A, Neil DM. 2014. Biophysical and biochemical changes  
494 occur in *Nephrops norvegicus* during starvation. *Journal of Experimental Marine Biology*  
495 *and Ecology* 457:81-9.
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497 Figure 1. Means ( $\pm$  SE) of A) copper concentration in the hepatopancreas (in relation to wet  
 498 weight); B) hepatosomatic index (HSI); C) lipid content of the hepatopancreas; D) water  
 499 content of the hepatopancreas in unfed (black bars) and fed (grey bars) groups of female  
 500 *Nephrops norvegicus* during the starvation trial. Letters indicate the results of a Tukey test  
 501 on the unfed group. Means that do not share a letter are significantly different ( $p < 0.05$ ).  
 502 Asterisks indicate a significant difference between fed and unfed groups at week 20 ( $p < 0.05$ )  
 503  $n=6$ .

504 Figure 2. Number of females at various ovary stages sampled during the aquarium trial.  
 505 White, Stage 0 (white ovaries); Light grey, Stage 1 (cream ovaries); Grey, Stage 2 (Pale  
 506 green ovaries); Black, Stage 3 (dark green ovaries); White with dots, Stage 4 (Dark green  
 507 and swollen eggs visible); White with hatching, Stage 5 mottled green reabsorbed) After  
 508 Tuck et al.(1997).

509 Figure 3. Means ( $\pm$  SE) of A) copper concentration in the hepatopancreas (in relation to  
 510 wet weight); B) hepatosomatic index (HSI); C) lipid content of the hepatopancreas; D)  
 511 water content of the hepatopancreas in field caught females (left panels) and male (right  
 512 panels) *Nephrops norvegicus* in 2009 (circles). With unfed (square with cross) and unfed  
 513 (square no cross) means  $\pm$  SE shown in each graph as a comparison, values derived from  
 514 this study (females) and from Watts et al. (2014) (males) shown.

515 Figure 4 A) Sex composition of the trawl catch from the Clyde Sea Area transect in each  
 516 month of 2009. White section of bar represents proportion of the catch which are immature  
 517 females and the black bars represent the proportion of the catch which are mature females ;  
 518 B) Percentage of females at each ovary maturation stage (see scale in Figure 2 legend). C)  
 519 Percentage of females that were *Ovigerousovigerous*.

520 Figure 5. Size distributions (according to carapace length) of (A) female and (B) male  
 521 *Nephrops norvegicus* in trawl catch from the Clyde Sea Area transect in each month of  
 522 2009. White (10-19.9mm), Light grey (20-29.9mm), Grey (30-39.9mm), Dark grey (40-  
 523 49.9mm), Black (50mm+). Black dashed line represents size at onset of maturity of females  
 524 (SOM=26 mm CL).

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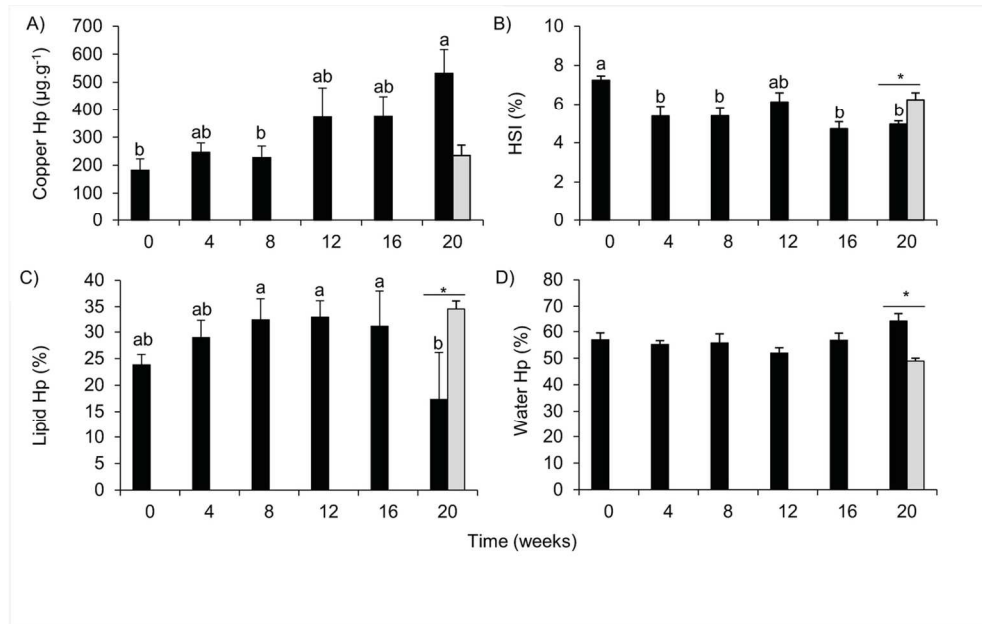


Figure 1. Means ( $\pm$  SE) of A) copper concentration in the hepatopancreas (in relation to wet weight); B) hepatosomatic index (HSI); C) lipid content of the hepatopancreas; D) water content of the hepatopancreas in unfed (black bars) and fed (grey bars) groups of female *Nephrops norvegicus* during the starvation trial. Letters indicate the results of a Tukey test on the unfed group. Means that do not share a letter are significantly different ( $p < 0.05$ ). Asterisks indicate a significant difference between fed and unfed groups at week 20 ( $p < 0.05$ )  $n=6$ .  
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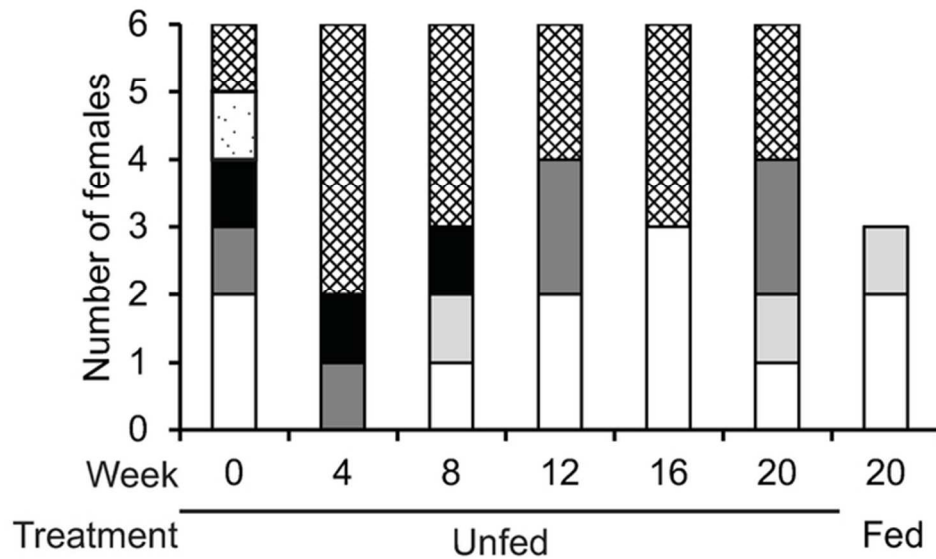


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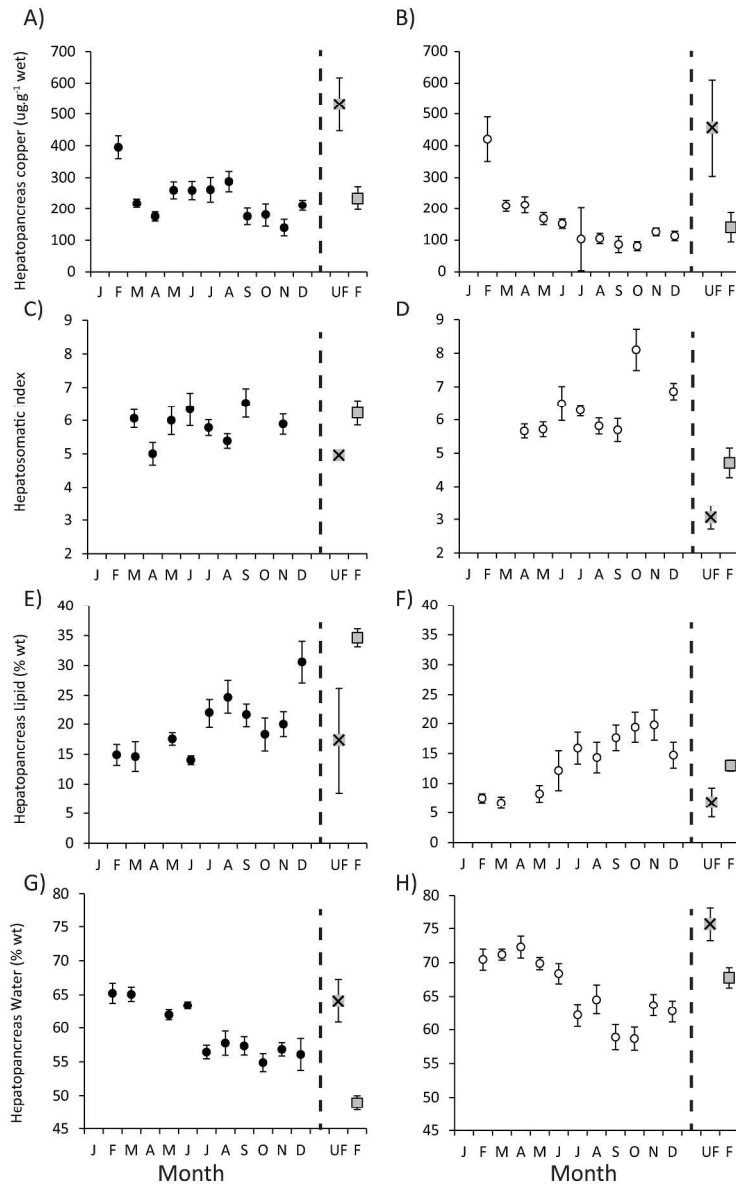


Figure 3. Means ( $\pm$  SE) of A) copper concentration in the hepatopancreas (in relation to wet weight); B) hepatopsomatic index (HSI); C) lipid content of the hepatopancreas; D) water content of the hepatopancreas in field caught females (left panels) and male (right panels) *Nephrops norvegicus* in 2009 (circles). With unfed (square with cross) and unfed (square no cross) means  $\pm$  SE shown in each graph as a comparison, values derived from this study (females) and from Watts et al. (2014) (males) shown.  
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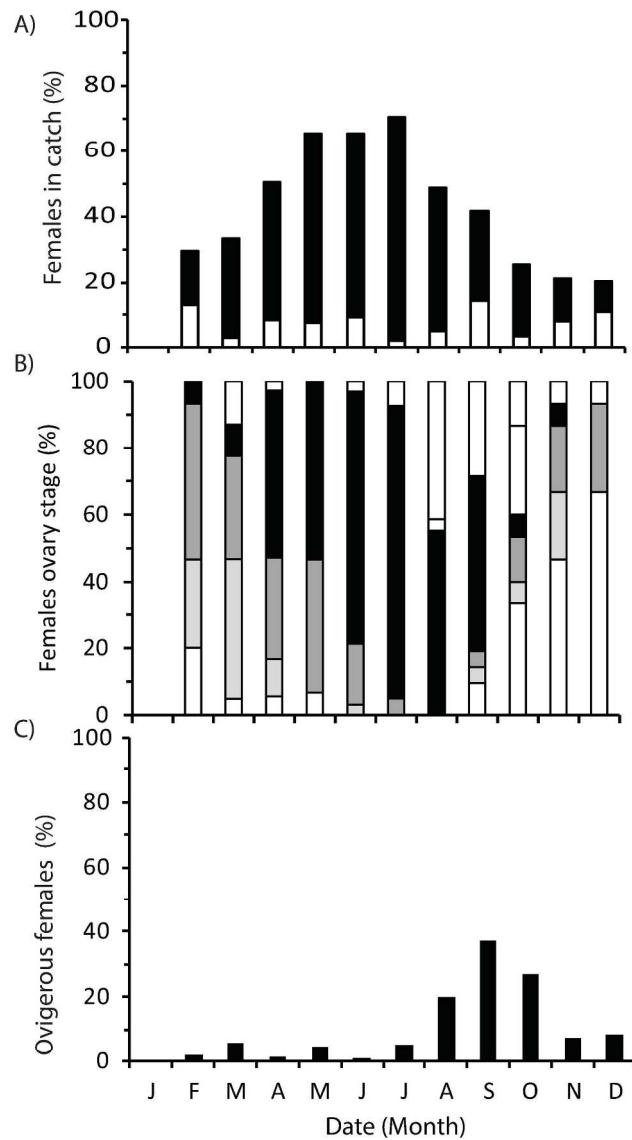


Figure 4 A) Sex composition of the trawl catch from the Clyde Sea Area transect in each month of 2009. White section of bar represents proportion of the catch which are immature females and the black bars represent the proportion of the catch which are mature females ; B) Percentage of females at each ovary maturation stage (see scale in Figure 2 legend). C) Percentage of females that were ovigerous. 166x307mm (300 x 300 DPI)

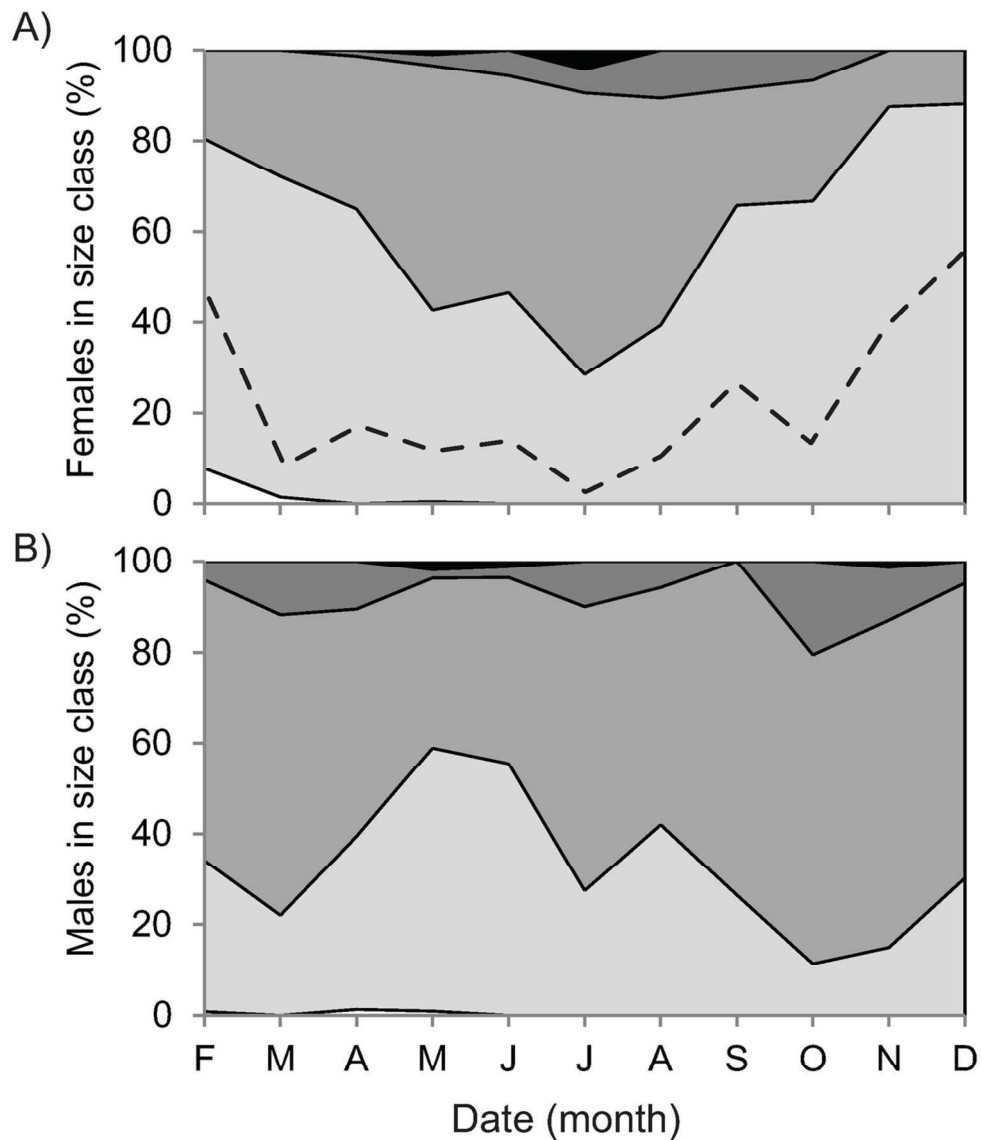


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106x127mm (300 x 300 DPI)