Marine Biology
Research
formerly Sarsia and Ophelia

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

SCHOLARONE™
Manuscripts

1   **Seasonal nutritional status in Norway lobsters, *Nephrops norvegicus* (L.): Are females**

2   **nutritionally compromised over the winter?**

3   Andrew JR WATTS*,[1a], Amaya ALBALAT,[1b], Ian P SMITH [2c], Robert JA ATKINSON [1,2],

4   Douglas M NEIL[1].

5   *[1]Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow,*

6   *Graham Kerr Building, Scotland, UK.*

7   *[2]University Marine Biological Station, Millport, Isle of Cumbrae, Scotland, UK.*

8   *[a] Department of Biosciences, College of Life and Environmental Sciences, University of*

9   *Exeter, Geoffrey Pope Building, Stocker Road, Exeter, EX4 4QD, United Kingdom*

10   *[b] School of Natural Sciences, University of Stirling, F12 Pathfoot building Stirling, FK9*

11   *4LA, United Kingdom*

12   *[c] School of Biological Sciences, University of Aberdeen, Zoology Building, Tillydrone Avenue,*

13   *Aberdeen, AB24 2TZ, United Kingdom*

14

15

16   ***Corresponding Author:** Andrew JR Watts

17   E-mail: A.watts.research@gmail.com

18

19   **Running head**

20   Seasonal nutritional status in lobster *N. norvegicus*

21

22

23

24

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

47

48

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   **Comment [a1]:** Comment1: addition

117 time for female *N. norvegicus* is it is entirely possible that they can also survive for this   **Comment [a2]:** Comment2: deletion

118 duration without feeding.

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 ± 2.78 to 2.58 ± 1.32
133   (wet weight), with a corresponding increase of water content from 68.05 ± 2.22% to 75.62 ±
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 ± 1.28 % in well fed *N.*
136   *norvegicus* and 13.35 ± 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

> **Comment [a3]:** Comment3: addition

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 ± 42.17 µg
143   g−1 to 423.37 ± 158.88 µg 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.

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 (± SD) carapace length of 33.2 mm ± 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 ± 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.

> **Comment [a4]:** Comment4: correction

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°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 $\delta^{15}N$, $\delta^{13}C$ and the carbon-nitrogen ratio (C:N), were made on

188 each animal, using methods described by Watts et al. (2014).

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 m ± 1.1 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°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 °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 δ^{13}C ($F_{5,35}$=2.03, *p*=0.103; $F_{1,8}$=1.63, *p*=0.243),

254   abdominal tail muscle δ^{15}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 ± 3.47%) in December. (Table SIII1A).

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 ± 69.81 µg.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 1B). 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% ±

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.

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

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

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 2ºC 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

412

413   <u>**References**</u>

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   Beevers 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.

496

497   Figure 1. Means (± SE) of A) copper concentration in the hepatopancreas (in relation to wet

498   weight); B) hepatopsomatic 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
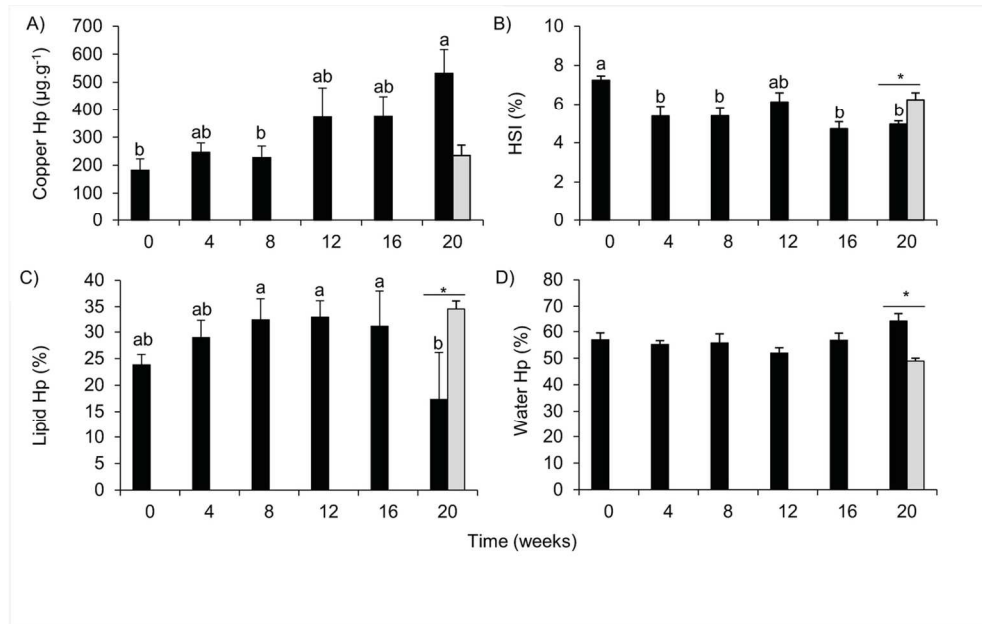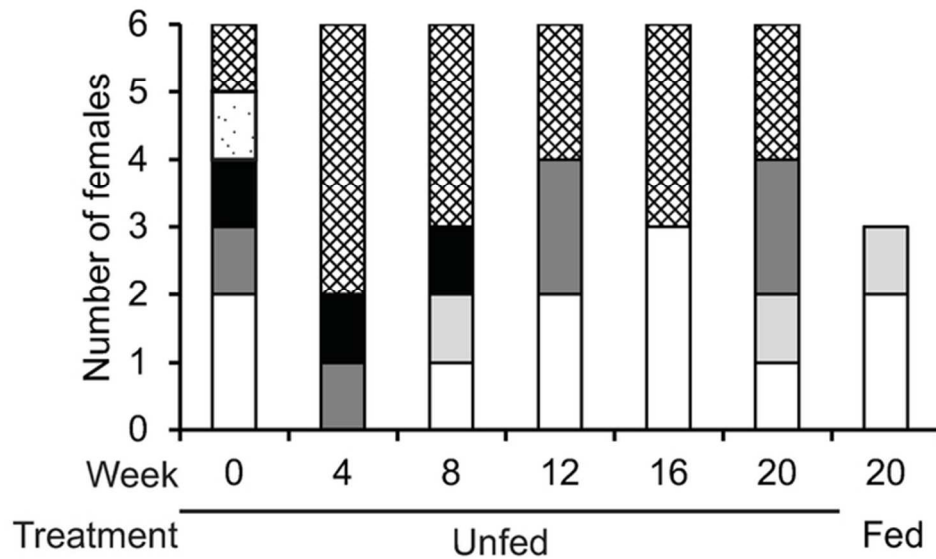
508   Tuck et al.(1997).

509   Figure 3. Means (± SE) of A) copper concentration in the hepatopancreas (in relation to

510   wet weight); B) hepatopsomatic 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 ± 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 ~~Ovigerous~~ovigerous.

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).

Figure 1. Means (± 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 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.
120x74mm (300 x 300 DPI)

Figure 2. Number of females at various ovary stages sampled during the aquarium trial. White, Stage 0 (white ovaries); Light grey, Stage 1 (cream ovaries); Grey, Stage 2 (Pale green ovaries); Black, Stage 3 (dark green ovaries); White with dots, Stage 4 (Dark green and swollen eggs visible); White with hatching, Stage 5 mottled green reabsorbed) After Tuck et al.(1997).
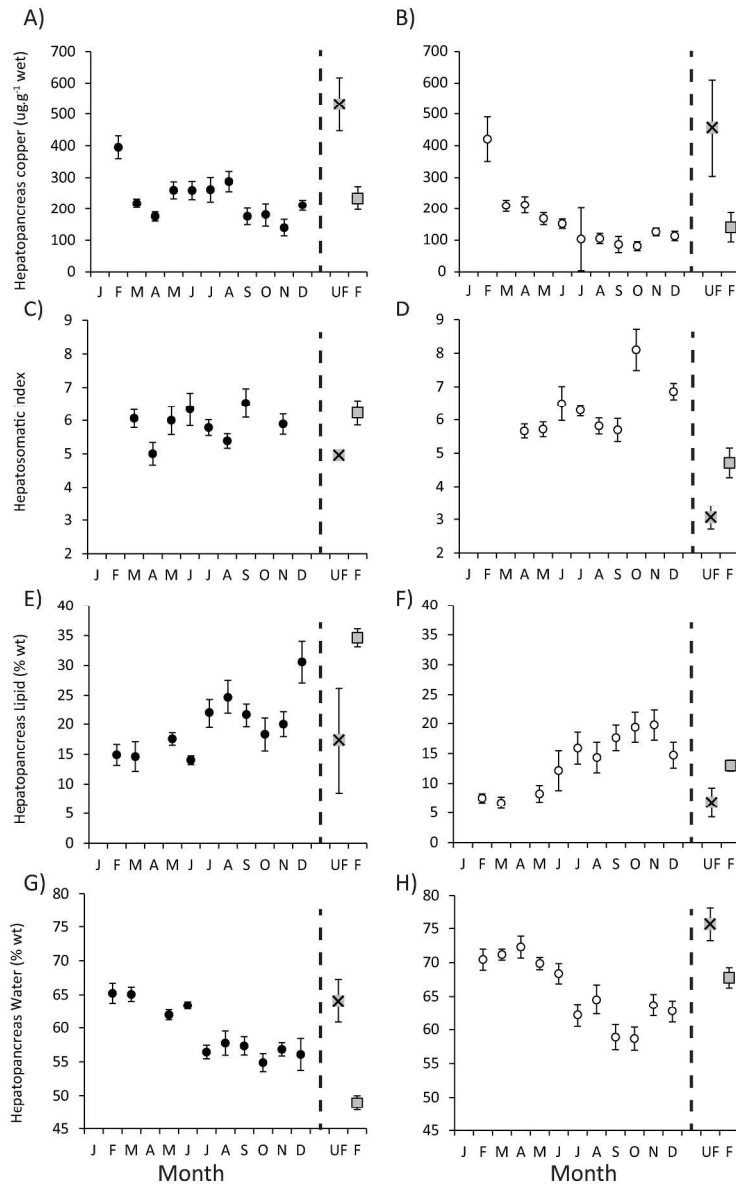60x37mm (300 x 300 DPI)

Figure 3. Means (± 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 ± SE shown in each graph as a comparison, values derived from this study (females) and from Watts et al. (2014) (males) shown.
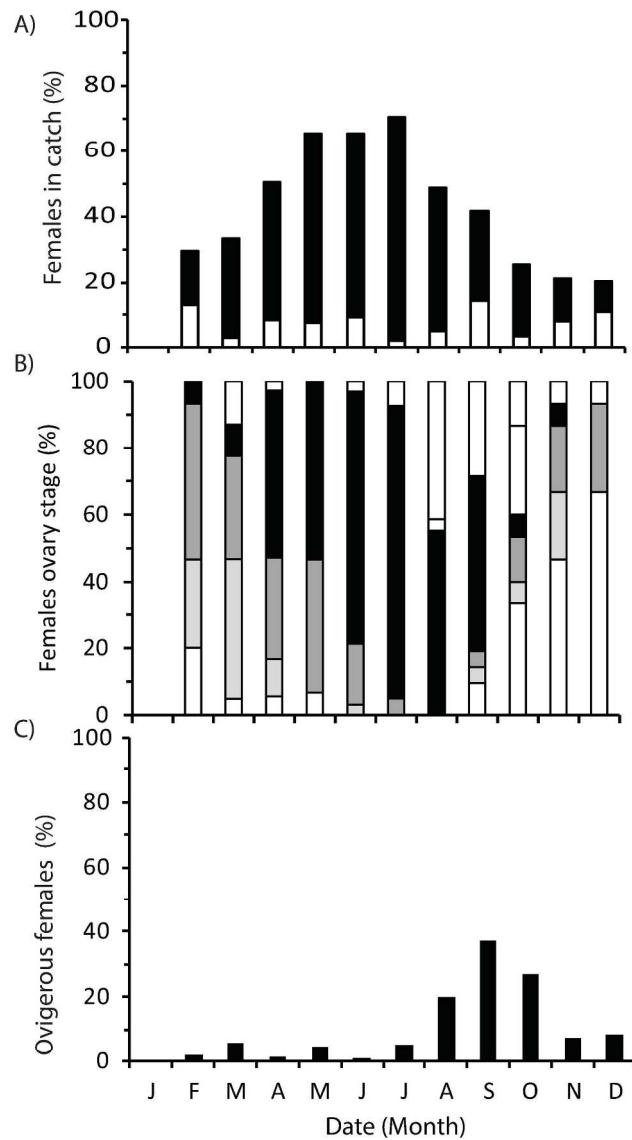283x342mm (300 x 300 DPI)

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.
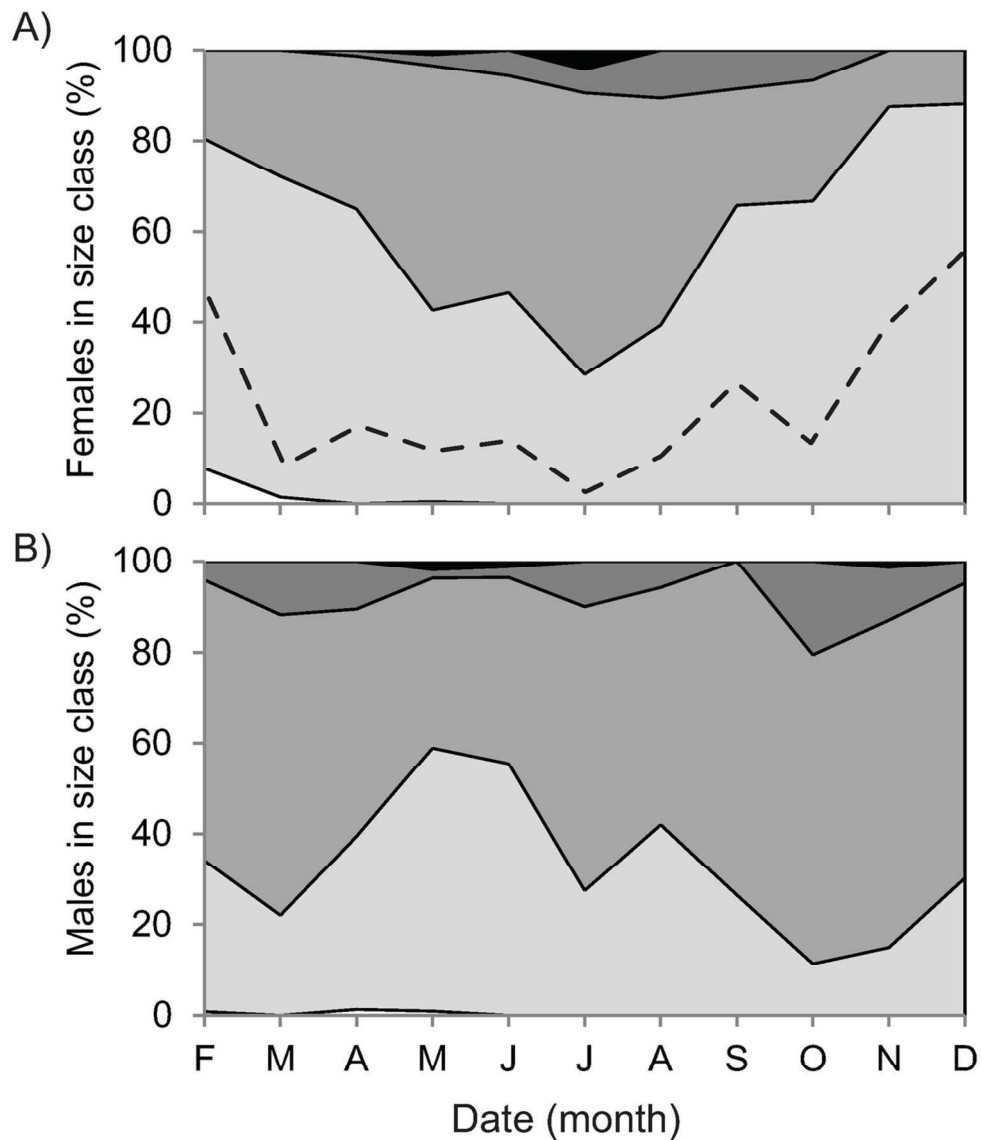166x307mm (300 x 300 DPI)

Figure 5. Size distributions (according to carapace length) of (A) female and (B) male *Nephrops norvegicus* in trawl catch from the Clyde Sea Area transect in each month of 2009. White (10-19.9mm), Light grey (20-29.9mm), Grey (30-39.9mm), Dark grey (40-49.9mm), Black (50mm+). Black dashed line represents size at onset of maturity of females (SOM=26 mm CL).
106x127mm (300 x 300 DPI)