

1 **Effect of starvation on the survival, injury and weight of adult snow crab,**  
2 ***Chionoecetes opilio***

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28 Keywords: Snow crab, *Chionoecetes opilio*, feeding, starvation, social interaction, animal welfare

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31 **Abstract**

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33 In this study, the effects of feeding (F) and starvation (S) on survival, injury and weight of captive  
34 male snow crab were examined. The experiment was carried out with three replicates of each  
35 treatment. Each replicate had 11 individually tagged crabs (average weight of  $650 \text{ g} \pm 37 \text{ S.E}$ ) per  
36 tank (replicate) in a total of 6 circular tanks (500 L), with stocking densities of  $14 \text{ kg m}^{-3}$ . The  
37 experiment ran for 100 days and the animals were exposed to ambient seawater temperatures ranging  
38 from 4.4 to 5.4°C during the trial period. There were no significant differences in wet weight between  
39 the groups at the start or at the end of the experiment. However, the hepatopancreas index (HI)  
40 significantly decreased in both groups from 6.1% at the start to respectively 4.6 % (F) and 3.2 % (S)  
41 at day 100. The S group had a significantly lower HI than the F group. Only one animal died during  
42 the experiment (day 100, F). The frequencies of injuries were similar in all groups. The results show  
43 that adult male snow crab can be stored for minimum 100 days (with or without feeding) with  
44 relatively low risk of physical injury, or mortality due to social interactions.

45

## 46 **Introduction**

47 The snow crab (*Chionoecetes opilio*) is an abundant and commercially important species in the Bering  
48 Sea, along the East coast of Canada and the West coast of Greenland (Lorentzen, Voldnes, Whitaker,  
49 Kvalvik, Vang, Gjerp Solstad, Thomassen & Siikavuopio 2018). The snow crab was first observed  
50 in the Barents Sea in 1996 (Kuzmin, Akhtarin & Menins 1999). However, the time and mode of  
51 colonization in the Barents Sea is unknown (Kuzmin et al., 1999; Pavlov & Sundet 2011).  
52 Commercial fishing for snow crab in the Barents Sea only started in the last few years but the fishery  
53 is rapidly growing (Dvoretzky & Dvoretzky 2015; Lorentzen et al. 2018), and is now permanently  
54 established in Norway. In 2016, the export of frozen clusters of snow crabs reached 3.952 tons, at a  
55 value of NOK 331 million (Lorentzen et al. 2018). After capture, the snow crab is either processed  
56 immediately or kept alive for periods ranging from 1 to 8 weeks (Siikavuopio, James, Olsen, Evensen,  
57 Mortensen & Olsen 2017) for subsequent live export. The live storage process includes both storage  
58 in water tanks and subsequent dry transport in polystyrene boxes to the destination market. This  
59 method enables the industry to control the time of processing and/or transport of live crabs. The  
60 further development of this live storage industry will rely on a reliable and consistent supply of crabs.  
61 Once this has been secured, storage and transport of crabs in good conditions to overseas markets is  
62 the next step (Lorentzen et al., 2018). In Norway, this is referred to as capture based aquaculture  
63 (CBA). CBA of snow crab is developing in Norway and effort has been put into establishing small-  
64 and medium sized enterprises using intensive holding systems for live holding of snow crabs  
65 (Siikavuopio et al. 2017; Lorentzen et al. 2018). Mortality rates during live storage depends on several  
66 biotic – and abiotic variables. The snow crab is more sensitive to high temperature and high stocking  
67 density under live storage compared to red king crab (Hardy, et al. 1994; Hardy, et al. 2000, James,  
68 et al. 2013; Siikavuopio, et al. 2014; Siikavuopio et al. 2017). Reducing the mortality of snow crab  
69 during capture and whilst it is being held in live storage is important for both economic and the  
70 welfare of the animal (Stoner 2012). Currently 99% of snow crab in Norway are processed as cooked  
71 and frozen clusters on board or on land based processing plants, while only 1% are exported live

72 (Lorentzen et al. 2018). The reason for the low live export is because the fishing boats are set up for  
73 capture and processing offshore. However, export of live snow crab is a more lucrative option as the  
74 price per kilo is about four times higher than that of clusters (Lorentzen et al. 2018). In addition, it is  
75 more favorable to be paid per kilo for the entire animal, rather than just the clusters, as by-products  
76 represent about 30% of the total snow crab weight (Lorentzen et al. 2018). The authors believe that  
77 due to a higher price for live crabs, the proportion of live snow crab exports will increase significantly.  
78 This was observed in the red king crab fisheries in Norway (Siikavuopio et al. 2014; Lorentzen et al.  
79 2018), this will rely on improved live holding methods and protocols being available for snow crabs.  
80 This is currently lacking for the snow crab industry (Dutil, Munro & Peloquin 1997; Siikavuopio &  
81 James, 2015; Siikavuopio *et al.* 2017). Thus, there is a need to develop and improve techniques for  
82 both short-term (vessel) and long-term (land) storage. In the case of red king crab, it has been  
83 demonstrated that increasing temperature and stocking densities increase the risk of cannibalism,  
84 mortality and injuries (Siikavuopio & James, 2015; Siikavuopio et al., 2014). In Norway, the red  
85 king crab and snow crab are currently starved during periods of live storage. For many species of  
86 crustacean starvation can lead to cannibalism and increased aggressiveness which in turn can cause  
87 poor animal welfare and reduced quality (Wickins & Lee 2002).

88 This study was therefore undertaken in order to describe the effects of feeding (F) and starvation (S)  
89 on survival, injuries and weight changes of wild caught male snow crab held in captivity for 100 days  
90 post capture.

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## 93 **Materials and methods**

### 94 *Experimental set up*

95 Mature male snow crabs (*C. opilio*) were caught by the vessel Northeastern (Opilio AS) on 10 January  
96 2017, using traditional snow crab pots in the NEAFC area (74.58 latitude and 38.49 longitude). The  
97 crabs were transported to the Aquaculture Research Station in Tromsø, Norway (~70°N), and placed  
98 in one 6 m<sup>3</sup> tank supplied with running seawater (~ 3.0°C, salinity ~ 32‰). The animal were not fed  
99 until the start of the experiment. At the start of the experiment, 60 crabs were individually measured  
100 and inspected for injuries before being randomly distributed into 6 circular tanks (500 L, 3 replicates)  
101 at a density of 17 kg m<sup>-3</sup>. The crabs were individually tagged with FT-69 tag (Floy-tag) glued to the  
102 carapace. The experiment was conducted from 20 January 2017 to 13 May 2017 (100 days). Each  
103 tank was supplied with filtered seawater (filtered to a nominal 150µm through a sand filter, UV-  
104 treated and with a salinity of 34 ‰) at a rate of 4 l min<sup>-1</sup> kg<sup>-1</sup>. All tanks were supplied with constant  
105 seawater with ambient temperature ranging from 4.4 to 5.4°C during the trial period. Water  
106 temperatures were recorded every day. Oxygen levels were measured with a Handy Delta logger  
107 (OxyGuard; OxyGard, International A/S, Blokken, Denmark) and were above 95% saturation in all  
108 tanks throughout the experiment. The crabs in the F group were fed *ad libitum* with squid twice a  
109 week for a period of 3 hours. Uneaten feed was siphoned and sieved from the tanks. Feed intake was  
110 calculated as the difference between the wet weight of the feed delivered and uneaten feed collected  
111 (g feed animal<sup>-1</sup> week<sup>-1</sup>). Specific growth rate (SGR) was calculated according to the equation:  $SGR$   
112  $= ((\ln W_2 - \ln W_1) / (t_2 - t_1)) * 100$ , where  $W_2$  and  $W_1$  is the living weight (kg) of the crab at day  $t_2$   
113 (day 100) and  $t_1$  (day 0) day respectively. Hepatopancreas index (HI) was calculated according to the  
114 equation:  $HI = (H/W) * 100$ , where H is the weight of hepatopancreas and W are the live weight of  
115 the crab. Presence and type of injury or missing legs were observed at day 0, 30, 60 and 100 during  
116 inspection and from images of individual crabs taken at these respective dates for subsequent analysis.  
117 A scoring system was established to assess crab injury from the images.

118

119 *Injury assessment*

120 To measure the injury status of F and S snow crab, a quantitative scoring system was established  
121 (Table 1). This scoring system was developed with 4 criteria of crab injuries (See Table 1 for details).

122 To assess if there were a relationship between injuries and crab anatomy each registered observation  
123 was sorted anatomically (Figure 1). The crab anatomy were divided in left and right and each limb  
124 and limb segment given a number for easier registration of injuries. An overview of the different  
125 damages/injuries present on the crab images from day 0 were used as a reference for the scoring  
126 system. All limbs were included in the scoring scheme. “Old” injuries were easily distinguishable  
127 with black spots of melanosis. New injuries were harder to observe in the images due to lack of black  
128 color, but easier when examining the live crab. Thus, pinching- and undefined injuries were not  
129 graded but counted. Figure 2 shows examples of injuries registered in the various criteria.

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131 *Statistical treatment*

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133 Statistical analyses were performed using SYSTAT v. 12 (Systat Software, Inc., USA). Possible  
134 differences between S and F groups in weight, SGR and HI, were analysed using ANOVA with  
135 distribution of data compared to normal distribution using Shapiro–Wilk test, and homogeneity  
136 checked using Levene’s test (Zar 1996). The chi square test were used to compare injury between the  
137 S and F group. Significance was assumed when  $P < 0.05$ . Data are presented as mean  $\pm$  standard  
138 error (SE).

139

140 **Results**

141 *Weight, feed intake and specific growth rate (SGR)*

142 In the current experiment, the average initial wet weight of the snow crab was 649 g (F) ( $\pm 38$ ) and  
143 651g (S) ( $\pm 39$ ) respectively. The average wet weight at the end of the experiment had increased to  
144 670 g ( $\pm 39$ ) (F) and to 653 g (S) ( $\pm 40$ ) ( $P=0.859$ ) (Fig 3). The average feed intake (g feed animal<sup>-1</sup>  
145 week<sup>-1</sup>) was 7.03 g ( $\pm 0.49$ ). There were no significant differences in weight between the groups at  
146 the start or at the end of the experiment (Fig 3.). The mean SGR of the F group was 0.03 (0.03) and  
147 S group was 0.01(0.006), but there were no significant differences between the groups ( $P = 0.492$ ).

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149 *Hepatopancreas index (HI)*

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151 At the start of the experiment, the HI was 6.1 (0.03). At the end the average had significantly  
152 decreased in the two groups to 4.6 (0.15) and 3.2 (0.05) respectively. The S group had a significantly  
153 lower HI than the F group ( $P=0.001$ ). Dark spots, i.e. melanosis, were observed on the crabs during  
154 the storage period in both groups.

155

156 *Mortality and frequency of injury*

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158 Only one animal died during the experiment (day 100, F). The frequencies of injuries were similar  
159 in all groups (Table 2), with a total of 9 (F) and 8 (S).

160 The most common observation were loss of pereopods (walking legs) either the whole limb or the  
161 merus, carpus, manus or dactyl (limb segments). Loss of limbs were seen in the shoulder joint,  
162 between the basis and the ischium.

163 The position of injuries to the crab limbs at day 0 showed a significantly (Chi square,  $P<0.05$ ) higher  
164 number of injuries at *merus* than any of the other leg segments (Figure 4).

165 Results from scoring of injuries during trial revealed no visible difference between the starved or the  
166 fed crabs. The crabs in the F group started out with 16.7 % having lost cheliped or pereopods, 23.3  
167 % had lost dactyl tips and 10 % had lost limb segments before arriving to the research station (Table  
168 3). The S crabs had 30 % having loss of limbs, 20 % lost dactyl tips and 23.3 % had lost limb  
169 segments. No crab lost their cheliped either before or during trial.



170 **Discussion**

171 The live export of snow crab is a new industry in Norway and it will rely on a consistent supply of  
172 wild caught crabs, held in land-based facilities for periods varying from a few days to months  
173 (Lorentzen, Rotabakk, Olsen, Skueland & Siikavuopio 2015, Lorentzen *et al.*, 2018). During this  
174 period, they will need to survive and maintain their quality at an acceptable level for both discerning  
175 export markets as well as to meet the stringent animal welfare standards in Norway.

176 Godbout *et al.* (2002) reported that snow crabs were able to survive long periods of food deprivation  
177 with low rates of mortality and no marked effects on body condition at low temperatures. Our study  
178 supports these results, with the loss of only one crab during the experimental period. Godbout *et al.*  
179 (2002) also reported that an overall improvement of nutritional condition occurred and the meat yield  
180 increased markedly in snow crabs held and fed in land-based facilities. However, in our study, there  
181 were no significant differences in wet weight of the crab between the F and S group at the end of the  
182 experiment.

183 In crustaceans, the hepatopancreas is generally regarded as a major lipid storage organ and during  
184 starvation, body fat especially from the hepatopancreas is metabolized (Wen Ku, Zhou 2006). This  
185 was the case in our study, where the hepatopancreas index was significant lower in the S group  
186 compared to the F group. It is worth noting that in the F group the hepatopancreas index was also  
187 significantly lower compared with the start value. A feed regime of squid fed twice per week for a  
188 period of 3 hours appears to be too low to maintain the hepatopancreas index of snow crab held in  
189 land-based facilities. The fact that there is difference in HI between F and S would indicate that F  
190 group were at least partially fed. However, the F treatment did have a lower HI at the conclusion of  
191 the experiment than the initial HI which would indicate that further work is required to describe  
192 optimal feed regimes for captive snow crab.

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195 The hepatopancreas is major lipid storage organ and squid muscle has relatively low lipid level, which  
196 may also explain the reduction in HI in our study (Wen *et al.* 2006).

197 There is a limited amount of information available on the impact of starvation in snow crab related to  
198 physiological and biochemical changes. Mayrand *et al.*, 2000 showed that 60-day period of food  
199 deprivation resulted in a reduction of muscle mass, DNA content, and enzyme activity in the merus  
200 muscle, and a reduction in size of the digestive gland, while gonad weight increased in male adult  
201 snow crab following terminal moult. Hardy *et al.*, (2000), illustrated that water content of merus  
202 muscle increased markedly over time, while protein and lipid content decreased over time. This  
203 should also be taken in mind evaluating the possible quality changes related to starvation and different  
204 feeding regimes of the snow crab. This was not included in the current study.

205 Both hunger and restriction in food availability can trigger aggression in animals held in land-based  
206 holding systems over time. The current study indicates snow crabs have the ability to endure long  
207 periods without feeding and during this time will show low territorial and/or aggressive behavior.  
208 This explains why we don't observe cannibalism and damage as a consequence of 100 days of  
209 starvation. There are relatively few studies focusing on possible negative social interaction of adult  
210 snow crab related to live holding conditions (e.g. stocking density and temperature) (Siikavuopio *et*  
211 *al.*, 2017).

212 In this study, the crabs started with 17 % having lost cheliped or pereopods, 23 % had lost dactyl  
213 tips and 10 % had lost limb segments. These losses may be due to autotomy of the leg after a claw  
214 bite to the affected leg. Autotomy is well documented in other crustacean as a means of avoiding  
215 predation or limiting the effects of wounds (Jaunes & Smith 1995; Siikavuopio *et al.*, 2017). The crab  
216 injury status was reasonably good at the beginning of the experiment and did not change considerably  
217 during the 100 days experimental period. Statistical analysis of pinching injury location was  
218 significantly higher in the leg segment merus. Most of these injuries was on the left side of the crab.  
219 The origin of these injuries can be related to events occurred before the catch or as a result of the  
220 capture and transport of the crab. The authors are unsure what the cause of the injuries incurred prior

221 to the experiment were, nor why there is differences between left and right side of the crab. More  
222 research is needed to evaluate how these injuries occur. Occasionally, dark spots, i.e. melanosis were  
223 observed on the crabs during the storage period. This observation is most probably due to a chemical  
224 defense mechanism, activated as part of a damage repair response (Vazquez, et al., 2009). The effector  
225 mechanisms include a coagulation cascade to avoid the loss of hemolymph and stimulation of the  
226 production of melanin by activating prophenoloxidase (Vargas-Albores & Yepis-Plascencia, 2000).  
227 It has been shown that for crustacean held in captivity cannibalism and increased aggressive behavior  
228 is also expected to occur more frequently as size heterogeneity increases (Dutil, et al., 1997). In the  
229 current study all crabs were in the same size group, and had the space available to sit in a single layer  
230 at the bottom of the tank. This may explain the low levels of aggressive behavior mortality in this  
231 experiments. Although, the crabs had the space available to be in a single layer at the bottom of the  
232 holding tanks in the current experiment, observations during the experimental period showed they  
233 chose to crowd together in stacks in small areas of the tank. This is a similar ‘crowding’ behavior to  
234 the red king crab (Siikavuopio et al., 2017). This supports the theory that the snow crab have low  
235 levels of territorial aggressiveness.

236 The current study has shown that adult (commercial size) snow crab can be held at 3°C for a minimum  
237 100 days, either being feed or starved, without significant risk of injury or death. The current  
238 experiment showed that the total wet weight of the crabs prior to and after the experiment were equal.  
239 However, the size of the hepatopancreas decreased, indicating that the crab are utilizing this organ  
240 during periods of starvation. More study is required, on possible changes in the meat quality as a  
241 result of these changes in metabolism. This may have a significant impact on the quality of the crab  
242 flesh and subsequently the value of the product.

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Table 1. Scoring system for crab injuries: Criteria 1 is based on loss of limbs, criteria 2 on the loss of limb tips, criteria 3 on the loss of carpus, manus or dactyls of the limbs, and criteria 4 on the number of damage originating from pinching and or other undefined injuries on the extremities.

<b>Criteria 1 - Loss of limbs</b>		
Score	Change	Appearance
1	No	All limb are intact
2	Mild	Possible beginning loss of limbs
3	Distinct	Leg/claw half way lost
4	Severe	Complete loss of leg/claw
<b>Criteria 2 - Loss of limb tips</b>		
Score	Change	Appearance
1	No	Tips are intact
2	Mild	Possible beginning loss of tip
3	Distinct	Tip half way lost
4	Severe	Complete loss of tip
<b>Criteria 3 - Loss of limbs carpus, manus or dactyls</b>		
Score	Change	Appearance
1	No	All limbs is intact
2	Mild	Possible beginning loss of limb segments. Seen as rift in joints between carpus, manus or dactyl
3	Distinct	Joint between limb sections half way lost
4	Severe	Complete loss of carpus, manus or dactyl
<b>Criteria 4 - Pinching or undefined injuries</b>		
Count	Pinching of cheliped or pereipods. Seen as cracks in the exoskeleton transvers of the limb	
Count	Undefined injury. Seen as cracks in the exoskeleton of limbs	

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326



327 Table 2. Total number of injuries during trial for feed or starved adult male snow crab.  
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<b>Day</b>	<b>0</b>	<b>30</b>	<b>60</b>	<b>100</b>
<b>Fed</b>	83	0	5	4
<b>Starved</b>	79	1	2	5

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331 **Table 3.** Results from scoring of snow crab limbs (%) and the number caused by aggressive  
 332 behavior between crabs (pinching) and undefined injuries. Injuries are related to category; No,  
 333 Mild, Distinct, and Severe, see table 1 for more information.  
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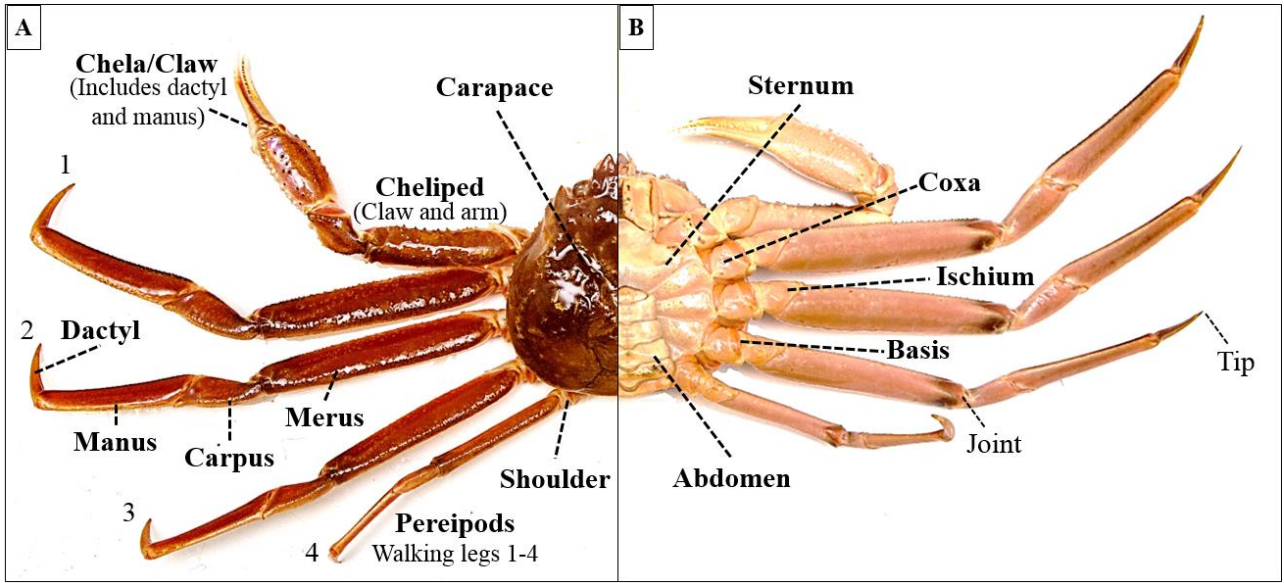
Group		Fed				Starved			
		0	30	60	100	0	30	60	100
Loss of legs	No	97,3	97,3	96,9	96,1	94,3	94,0	93,7	93,3
	Mild	1,0	0,0	0,0	0,0	2,0	0,0	0,0	0,0
	Distinct	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
	Severe	1,7	0,0	0,3	0,7	3,7	0,3	0,3	0,3
	Injured crabs	16,7	0,0	3,4	7,1	30,0	3,3	3,3	3,3
	Whole crabs	83,3	83,3	79,3	71,4	70,0	66,7	63,3	60,0
Loss of dactyl tips	No	94,3	94,3	93,4	93,2	96,0	96,0	96,0	95,3
	Mild	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
	Distinct	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
	Severe	5,7	0,0	0,7	0,0	4,0	0,0	0,0	0,7
	Injured crabs	23,3	0,0	6,9	0,0	20,0	0,0	0,0	3,3
	Whole crabs	76,7	76,7	69,0	67,9	80,0	80,0	80,0	76,7
Loss of limb segments	No	99,7	99,7	99,7	99,6	99,5	99,5	99,5	99,5
	Mild	0,2	0,0	0,0	0,0	0,4	0,0	0,0	0,0
	Distinct	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1
	Severe	0,1	0,0	0,1	0,0	0,1	0,0	0,0	0,0
	Injured crabs	10,0	0,0	3,4	0,0	23,3	0,0	0,0	3,3
	Whole crabs	90,0	90,0	86,2	85,7	76,7	76,7	76,7	73,3
Pinching injuries	Amount	19	0	0	0	11	0	1	3
	Injured crabs	11	0	0	3	7	0	1	2
	Unharmed crabs	19	19	18	14	23	23	22	20
Undefined injuries	Amount	35	0	1	0	32	0	0	0
	Injured crabs	6	0	1	0	12	0	0	0
	Unharmed crabs	24	24	22	21	18	18	18	18

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338 **Figure 1.** Snow crab anatomy. Figure shows top (A) and bottom (B) of the crab left side.

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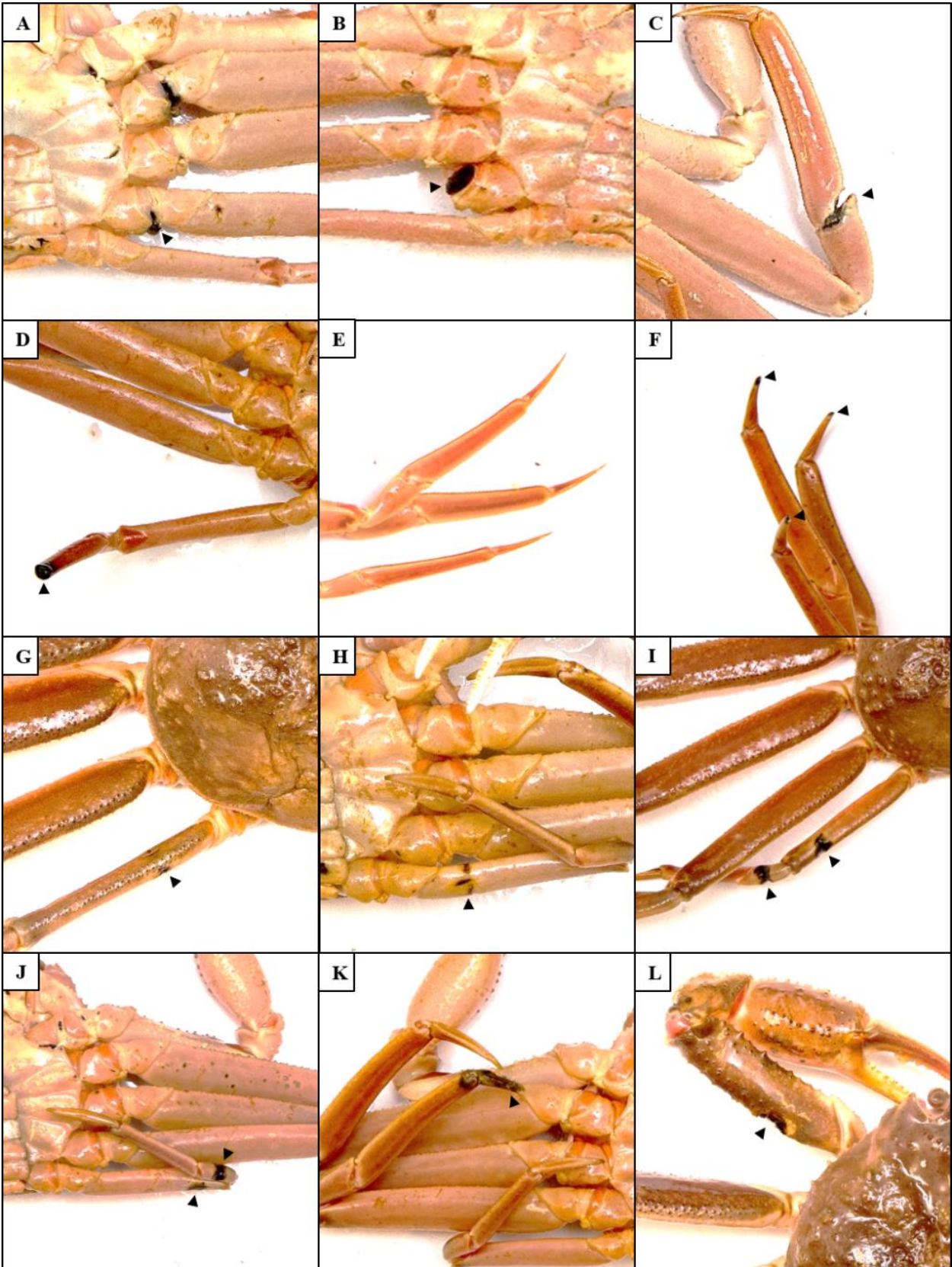


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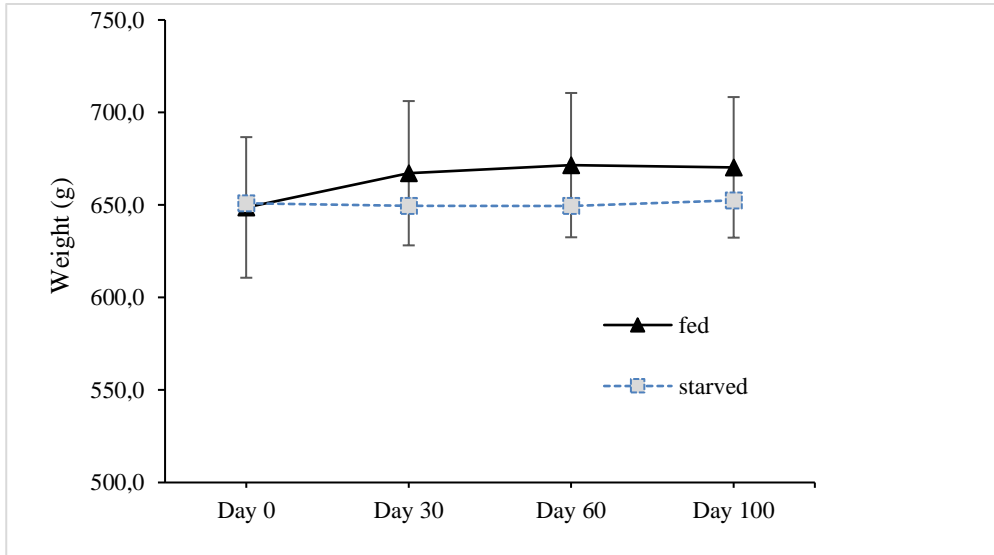
343 **Figure 2.** Injury criteria one to four – Loss of limbs, carpuses, manuses, dactyls or tips, pinching  
344 injuries and undefined injuries seen in starved or fed Snow crab. (A) Possibly beginning loss of limb  
345 at the shoulder joint in pereipod number one and three, score 2 and 1. (B) Complete loss of pereipod  
346 number three, score 4. (C) Beginning loss of manus in left pereipod number one, score 3. (D)  
347 Complete loss of manus in leg number four, score 4. (E) Intact limb tips, score 1. (F) Tips broken off,  
348 score 4. Welfare criteria four – (G + H) Miner pinching injury seen from top (G) and bottom (H) of  
349 merus of pereipod number four. (I + J) Two major, assumed to be, pinching injuries on merus and  
350 carpus of left pereipod number four, seen from top (I) and bottom (J) of the crab. (K) Injury or damage  
351 to the dactyl of right pereipod number three, counted as one in the scoring scheme. (L) Injury to the  
352 merus of cheliped.



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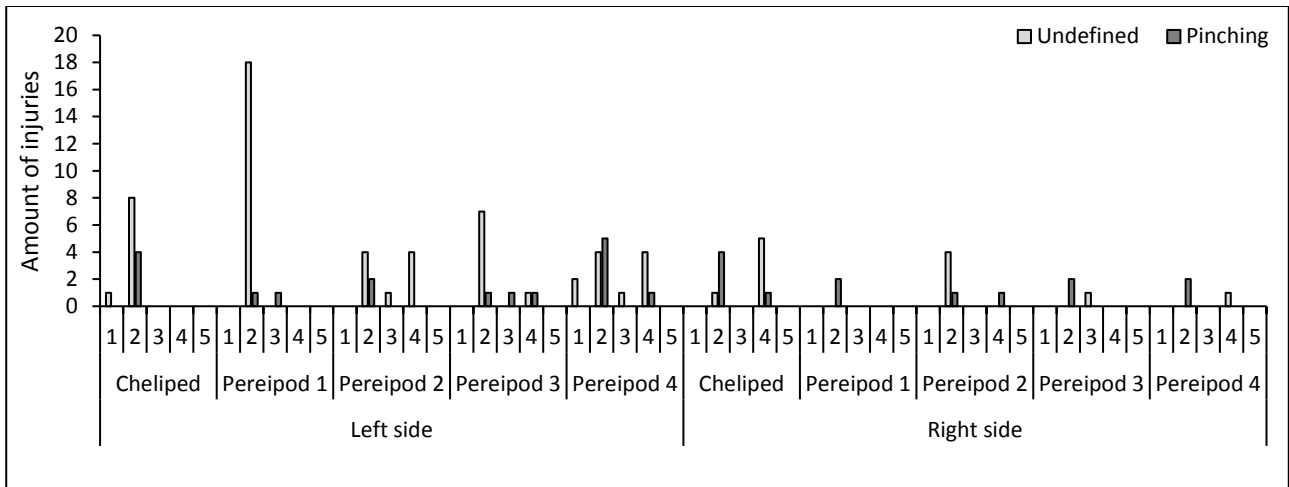
356 Figure 3. Average wet weight (g) of adult male snow crab in the Fed and Starved treatments at day  
357 0, 30, 60 and 100. Data is presented as mean  $\pm$  standard error

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364 Figure 4. Pinching- and undefined injuries registered at day 0. Registration of injuries shows a higher frequency of on the  
 365 meruses of the limbs than on the other four limb parts. Limb segments shown as numbers, 1: Shoulder, 2: *Merus*, 3:  
 366 *Carpus*, 4: *Manus* and 5: *Dactyl*.



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