Received: 26 April 2018 | Revised: 30 October 2018 | Accepted: 11 November 2018

DOI: 10.1111/are.13926

ORIGINAL ARTICLE

WILEY Aquaculture Research

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

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Funding information
NFR; FHF; Norwegian Seafood Research Fund; Norwegian Research Council; Nofima

Abstract

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

KEYWORDS
animal welfare, *Chionoecetes opilio*, feeding, snow crab, social interaction, starvation

1 | INTRODUCTION

The snow crab (*Chionoecetes opilio*) is an abundant and commercially important species in the Bering Sea, along the East coast of Canada and the West coast of Greenland (Lorentzen et al., 2018). The snow crab was first observed in the Barents Sea in 1996 (Kuzmin, Akhtarin, & Menins, 1999). However, the time and mode of colonization in the Barents Sea is unknown (Kuzmin et al., 1999; Pavlov & Sundet, 2011). Commercial fishing for snow crab in the Barents Sea only started in the last few years but the fishery is rapidly growing (Dvoretsky & Dvoretsky, 2015; Lorentzen et al., 2018), and is now permanently established in Norway. In 2016, the export of frozen clusters of snow crabs reached 3.952 tons, at a value of NOK 331 million (Lorentzen et al., 2018). After capture, the snow crab is either processed immediately or kept alive for periods ranging from 1 to 8 weeks (Siikavuopio et al., 2017) for subsequent live export. The live storage process includes both storage in water tanks and subsequent dry transport in polystyrene boxes to the destination market. This method enables the industry to control the time of processing and/or transport of live crabs. The further development of this live storage industry will rely on a reliable and consistent supply of crabs. Once this has been secured, storage and transport of crabs in good conditions to overseas markets is the next step (Lorentzen et al., 2018). In Norway, this is referred to as capture based aquaculture. Capture based aquaculture of snow crab is developing in Norway and effort has been put into establishing small- and medium sized enterprises using intensive holding systems for live holding of snow crabs (Lorentzen et al., 2018; Siikavuopio, et al., 2017). Mortality rates during live storage depend on several biotic—and abiotic variables. The snow crab is more sensitive to high temperature and high stocking density under live storage compared with red king crab (Hardy, Dutil, Godbout, & Munro, 2000; Hardy, Munro, & Dutil, 1994; James, Vasilyev, Siikavuopio, Kovatcheva, & Samuelsen, 2013; Siikavuopio et al., 2017; Siikavuopio, James, Olsen, Evensen, & Mortensen, 2014). Reducing the mortality of snow crab during capture and whilst it is being held in live storage is important for both economic and the welfare of the animal (Stoner, 2012).Currently 99% of snow crab in Norway are processed as cooked and frozen clusters on board or on land based processing plants, while only 1% are exported live (Lorentzen et al., 2018). The reason for the low live export is because the fishing boats are set up for capture and processing offshore. However, export of live snow crab is a more lucrative option as the price per kilo is about four times higher than that of clusters (Lorentzen et al., 2018). In addition, it is more favourable to be paid per kilo for the entire animal, rather than just the clusters, as by-products represent about 30% of the total snow crab weight (Lorentzen et al., 2018). The authors believe that due to a higher price for live crabs, the proportion of live snow crab exports will increase significantly. This was observed in the red king crab fisheries in Norway (Loretnzen et al., 2018; Siikavuopio et al., 2014), this will rely on improved live holding methods and protocols being available for snow crabs. This is currently lacking for the snow crab industry (Dutil, Munro, & Peloquin, 1997; Siikavuopio & James, 2015; Siikavuopio et al., 2017). Thus, there is a need to develop and improve techniques for both short-term (vessel) and long-term (land) storage. In the case of red king crab, it has been demonstrated that increasing temperature and stocking densities increase the risk of cannibalism, mortality, and injuries (Siikavuopio & James, 2015; Siikavuopio et al., 2014). In Norway, the red king crab and snow crab are currently starved during periods of live storage. For many species of crustacean starvation can lead to cannibalism and increased aggressiveness which in turn can cause poor animal welfare and reduced quality (Wickins & Lee, 2002).

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

2 | MATERIALS AND METHODS

2.1 | Experimental set-up

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

2.2 | Injury assessment

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

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 carpuses, manuses or dactyls of the limbs, and criteria 4 on the number of damage originating from pinching and or other undefined injuries on the extremities

Score	Change	Appearance
Criteria 1 – Loss of limbs		
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
Criteria 2 – Loss of limb tips		
1	No	Tips are intact
2	Mild	Possible beginning loss of tip
3	Distinct	Tip half way lost
4	Severe	Complete loss of tip
Criteria 3 – Loss of limbs: carpuses, manuses, or dactyls		
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
Criteria 4 – Pinching or undefined injuries		
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	

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

2.3 | Statistical treatment

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

3 | RESULTS

3.1 | Weight, feed intake, and specific growth rate

In the current experiment, the average initial wet weight of the snow crab was 649 g (F) (±38) and 651 g (S) (±39) respectively. The average wet weight at the end of the experiment had increased to 670 g (±39) (F) and to 653 g (S) (±40) ($p = 0.859$) (Figure 3). The average feed intake (g feed animal^{-1} week^{-1}) was 7.03 g (±0.49). There were no significant differences in weight between the groups at the start or at the end of the experiment (Figure 3.). The mean SGR of the F group was 0.03 (0.03) and S group was 0.01 (0.006), but there were no significant differences between the groups ($p = 0.492$).

3.2 | Hepatopancreas index

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

3.3 | Mortality and frequency of injury

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

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

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

Results from scoring of injuries during trial revealed no visible difference between the starved or the fed crabs. The crabs in the F

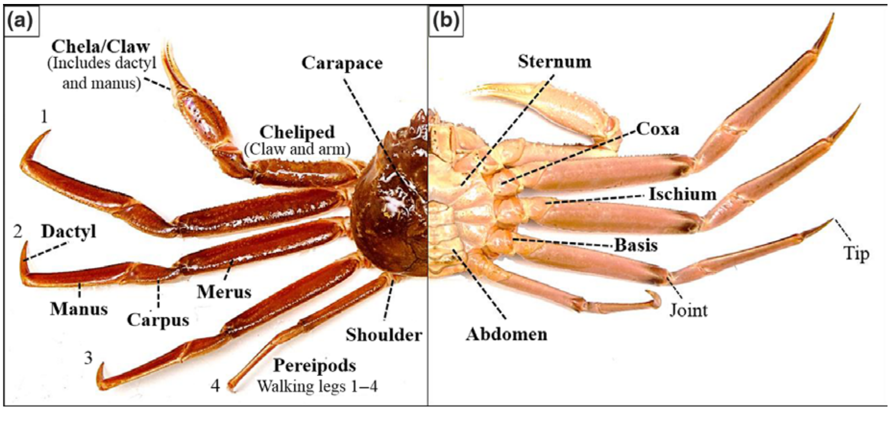
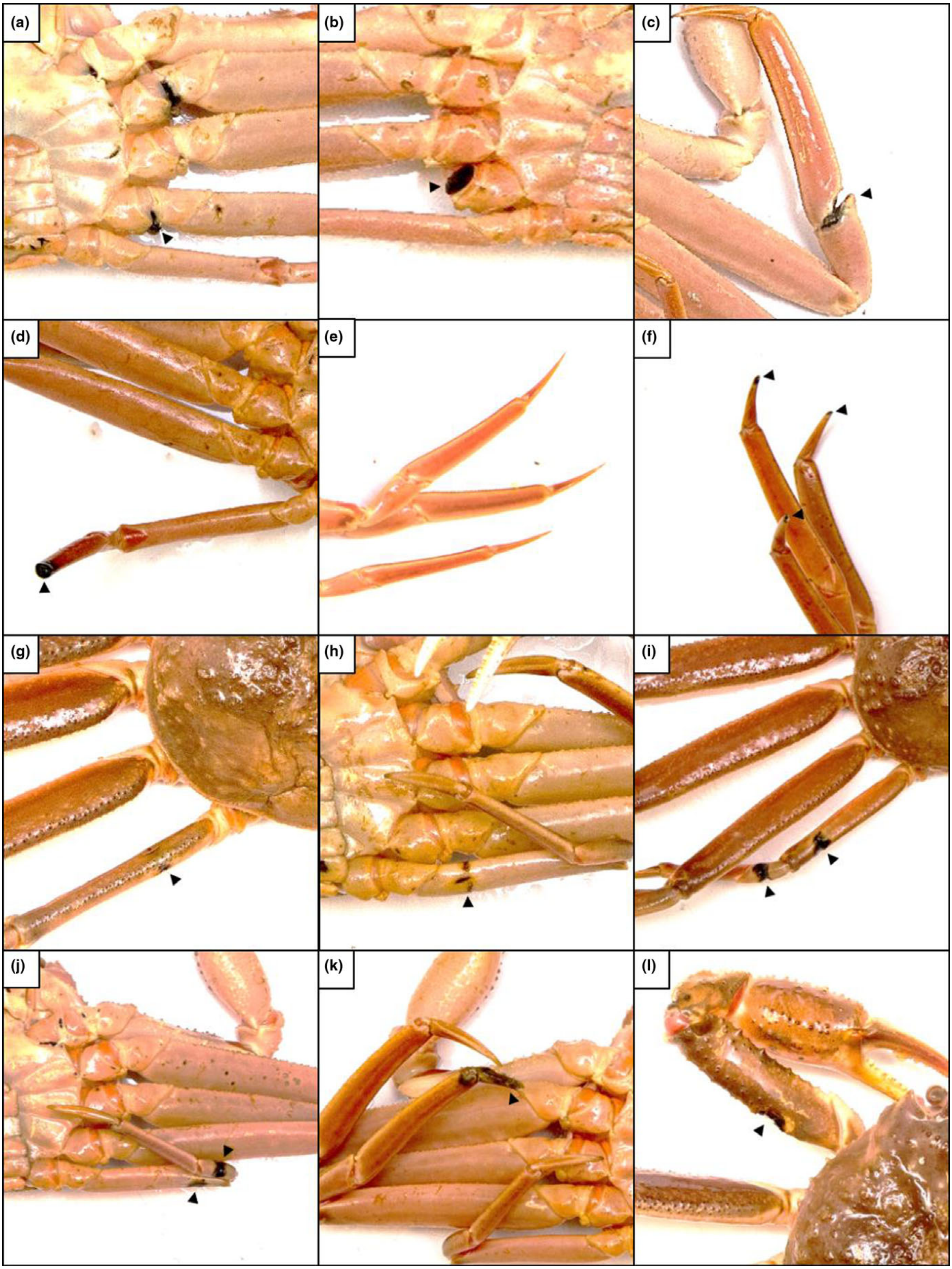


FIGURE 1 Snow crab anatomy. Figure shows top (a) and bottom (b) of the crab left side [Colour figure can be viewed at wileyonlinelibrary.com]

FIGURE 2 Injury criteria one to four – Loss of limbs, carpuses, manuses, dactyls or tips, pinching injuries and undefined injuries seen in starved or fed Snow crab. (a) Possibly beginning loss of limb at the shoulder joint in pereipod number one and three, score 2 and 1. (b) Complete loss of pereipod number three, score 4. (c) Beginning loss of manus in left pereipod number one, score 3. (d) Complete loss of manus in leg number four, score 4. (e) Intact limb tips, score 1. (f) Tips broken off, score 4. Welfare criteria four – (g + h) Miner pinching injury seen from top (g) and bottom (h) of merus of pereipod number four. (i + j) Two major, assumed to be, pinching injuries on merus and carpus of left pereipod number four, seen from top (i) and bottom (j) of the crab. (k) Injury or damage to the dactyl of right pereipod number three, counted as one in the scoring scheme. (l) Injury to the merus of cheliped [Colour figure can be viewed at wileyonlinelibrary.com]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group started out with 16.7% having lost cheliped or pereiopods, 23.3% had lost dactyl tips and 10% had lost limb segments before arriving to the research station (Table 3). The S crabs had 30% having loss of limbs, 20% lost dactyl tips and 23.3% had lost limb segments. No crab lost their cheliped either before or during trial.

4 | DISCUSSION

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

Godbout, Dutil, Hardy, and Munro (2002) reported that snow crabs were able to survive long periods of food deprivation with low

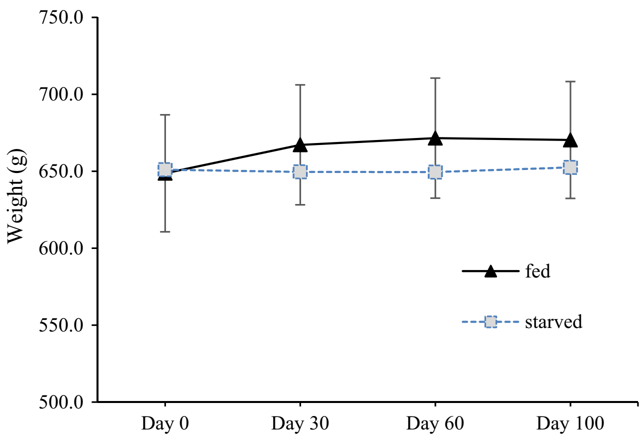

FIGURE 3 Average wet weight (g) of adult male snow crab in the Fed and Starved treatments at day 0, 30, 60 and 100. Data is presented as mean standard error [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Total number of injuries during trial for fed or starved adult male snow crab

Day	0	30	60	100
Fed	83	0	5	4
Starved	79	1	2	5

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

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

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

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

Both hunger and restriction in food availability can trigger aggression in animals held in land-based holding systems over time. The current study indicates that snow crabs have the ability to endure long periods without feeding and during this time will show low territorial and/or aggressive behaviour. This explains why we do

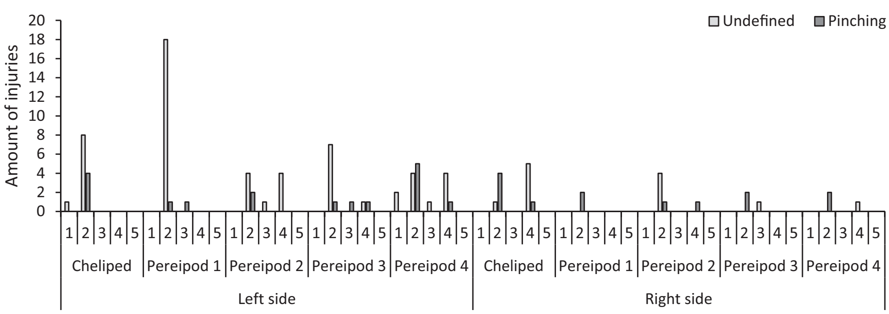

FIGURE 4 Pinching- and undefined injuries registered at day 0. Registration of injuries shows a higher frequency of on the meruses of the limbs than on the other four limb parts. Limb segments shown as numbers, 1: Shoulder, 2: *Merus*, 3: *Carpus*, 4: *Manus* and 5: *Dactyl*

TABLE 3 Results from scoring of snow crab limbs (%) and the number caused by aggressive behaviour between crabs (pinching) and undefined injuries. Injuries are related to category; No, Mild, Distinct, and Severe, see Table 1 for more information

Group	Fed				Starved			
Day	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

not observe cannibalism and damage as a consequence of 100 days of starvation. There are relatively few studies focusing on possible negative social interaction of adult snow crab related to live holding conditions (e.g. stocking density and temperature) (Siikavuopio et al., 2017).

In this study, the crabs started with 17% having lost cheliped or pereiopods, 23% had lost dactyl tips and 10% had lost limb segments. These losses may be due to autotomy of the leg after a claw bite to the affected leg. Autotomy is well-documented in other crustacean as a means of avoiding predation or limiting the effects of wounds (Jaunes & Smith, 1995; Siikavuopio et al., 2017). The crab injury status was reasonably good at the beginning of the experiment and did not change considerably during the 100 days experimental period. Statistical analysis of pinching injury location was significantly higher in the leg segment merus. Most of these injuries were on the left side of the crab. The origin of these injuries can be related to events occurred before the catch or as a result of the capture and transport of the crab. The authors are unsure what the cause of the injuries incurred prior to the experiment were, nor why there are differences between left and right side of the crab. More research is needed to evaluate how these injuries occur. Occasionally, dark spots, that is, melanosis were observed on the crabs during the storage period. This observation is most probably due to a chemical defense mechanism, activated as part of a damage repair response (Vazquez, et al., 2009). The effector mechanisms include a coagulation cascade to avoid the loss of hemolymph and stimulation of the production of melanin by activating prophenoloxidase (Vargas-Albores & Yepis-Plascencia, 2000). It has been shown that for crustacean held in captivity cannibalism and increased aggressive behaviour is also expected to occur more frequently as the size heterogeneity increases (Dutil, et al., 1997). In the current study all crabs were in the same size group, and had the space available to sit in a single layer at the bottom of the tank. This may explain the low levels of aggressive behaviour mortality in these experiments. Although, the crabs had the space available to be in a single layer at the bottom of the holding tanks in the current experiment, observations during the experimental period showed that they chose to crowd together in stacks in small areas of the tank. This is a similar "crowding" behaviour to the red king crab (Siikavuopio et al., 2017). This supports the theory that the snow crab has low levels of territorial aggressiveness.

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

ACKNOWLEDGMENTS

This project has been financed by the Norwegian Seafood Research Fund (FHF, Project no. 901254), NFR and Norwegian Research Council (SnowMap, project no. 267763) and Nofima. The authors thank the research station in Kårvika for their assistance throughout this experiment.

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How to cite this article: Siikavuopio SI, Johansson GS, James P, Lorentzen G. Effect of starvation on the survival, injury, and weight of adult snow crab, *Chionoecetes opilio*. *Aquac Res*. 2019;50:550–556. https://doi.org/10.1111/are.13926