Combining data from different sampling methods to study the development of an alien crab 1 2 Chionoecetes opilio invasion in the remote and pristine Arctic Kara Sea 3 Anna Konstantinovna Zalota<sup>1</sup>, Olga Leonidovna Zimina<sup>2</sup>, Vassily Albertovich 4 5 Spiridonov<sup>1</sup> 6 7 <sup>1</sup> Shirshov Institute of Oceanology, Russian Academy of Sciences (SIO RAS), Moscow, 8 Russia 9 <sup>2</sup> Murmansk Marine Biological Institute KSC, Russian Academy of Sciences (MMBI KSC 10 RAS), Murmansk, Russia 11 12 Corresponding Author: 13 Anna Zalota 14 36 Nakhimovskiy pr., Moscow, 117997, Russia 15 Email address: azalota@gmail.com 16 17 **Abstract** 18 Data obtained using three different sampling gearsgear is compared and combined to 19 assess the size composition and density of the non-indigenous snow crab population 20 Chionoecetes opilio in the previously free of alien species Kara Sea benthos. previously 21 practically free of introduced species. The Sigsbee trawl has small mesh and catches even 22 recently settled crabs. The large bottom trawl is able to catch large crabs, but does not retain 23 younger crabs, due to its large mesh. We used <u>Yv</u>ideo sampling <del>allows us</del> to observe larger crabs 24 although some smaller crabs can also be spotted. The combined use of these such gears gear could 25 provide full scope data of the existing size groups in a population. 26 The Delensity of the crabs has been was calculated from the video footage. The highest 27 figures were in Blagopoluchiya Bay at 0.87 crabs/m<sup>2</sup>, where the settlement seems to reach its first 28 peak of population growth after the introduction. High density i-and-in the Kara Gates Strait atat 29 0.87 and 0.55 crabs/m<sup>2</sup>, which could be due to the close proximity of the Barents Sea from where 30 the crabs can enter by both larvae dispersal and active adult migration-erabs/m<sup>2</sup> respectively.- All

size groups have been present in most sampled areas, which suggest successful settlement and

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growth of crabs over a number of years. Again, this was not the case in Blagopoluchiya Bay with high density of small crabs (<30 mm CW), which confirms its recent population growth, and in Haug Bay with very few crabs. Male to female ratio was strikingly different between the bays of the Novaya Zemlya Archipelago and west of the Yamal Peninsula (0.8 and 3.8 respectively). Seventy five ovigerous females were caught in 2016, which confirms the presence of a reproducing population in the Kara Sea. All size groups (instars) have been present in most sampled areas. This was not the case in Blagopoluchiya Bay, with high density of small crabs (<30 mm CW), and in Haug Bay with very few crabs.

The spatial and functional structure of the snow crab population in the Kara Sea is still in the process of formation. The presented data indicates that this process may lead to a complex

 The spatial and functional structure of the snow crab population in the Kara Sea is still in the process of formation. The presented data indicates that this process may lead to a complex system, which is based on local recruitment and transport of larvae from the Barents Sea and across the western Kara shelf; formation of nursery grounds; active migration of adults and their concentration in the areas of the shelf with appropriate feeding conditions.

#### INTRODUCTION

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48 The process of a non-indigenous species (NIS) invasion and naturalization can take a very Formatted: Highlight 49 long time (Byers et al., 2015). This is especially expected for a large predatory species that takes 50 years to achieve sexual maturity, such as crabs Chionoecetes opilio. However, for a biologist and 51 an ecologist, these are most interesting times. An ongoing invasion can expose the initial 52 structure of an ecosystem; its response to an invader can reveal its resistance and resilience 53 capacity; and the invader's process of acclimatization can manifest its biological and ecological 54 features that were less apparent in stable conditions. In this respect, anAn ongoing invasion of a 55 large predatory snow crab, *Chionoecetes opilio*, into the previously pristine Kara Sea benthic 56 environment is an exceptional opportunity for science. Due to the warming climate, and the Formatted: Highlight Formatted: Highlight 57 current decreasing sea ice cover in the Arctic, it is widely expected that the shipping and other 58 human activity will greatly increase (Ho, 2010; Liua and Kronbak, 2010). Such productive and 59 commercially important seas as the Bering and the Barents are very well studied and have 60 ongoing large scientific projects to study their crabs populations, such as within joint studies of Formatted: Highlight Russian-Norwegian studies of the Barents Sea Institute of Marine Research (IMR) and the 61 62 Knipovich Polar Institute of Fishery and Oceanography (PINRO) (Pavlov & Sundet, 2011; 63 Hammer & Hoel, 2012; Jørgensen et al., 2015; Sokolov et al., 2016). This is not the case for the 64 Siberian seas that have lower productivity, and until recently had very short ice-free seasons 65 (Zenkevich, 1963; Vinogradov et al., et al., 2000; AARI, 2009; Demidov & Mosharov, 2015; AARI, 66 2009). Biological research in the Kara Sea benthos is mostly conducted by occasional expeditions 67 of the Shirshov Institute of Oceanology, Russian Academy of Sciences (SIO)SIO RAS, 68 Murmansk Marine Biological Institute, Russian Academy of Sciences (MMBI)MMBI (see 69 authors' affiliation) and the Knipovich Polar Institute of Fishery and Oceanography (PINRO) 70 PINRO (Jørgensen et al., 1999; Sokolov et al., 2016). The intensity and area of sampling 71 effort varies, and so does the sampling gear employed. Therefore, it is important to compare and 72 understand the differences in the results obtained by different gear to be able to gather 73 informative data in the future and to understand the present situation. Formatted: Highlight 74 The snow crab, Chionoecetes opilio (Decapoda: Oregonidae) is one of the few 75 contemporary Arctic non-indigenous species (NIS), and it has invaded vast areas of the Barents Formatted: Highlight

and Kara seas with an unprecedented speed for a shelf species (Pavlov, 2006; Pavlov & Sundet,

78 Zalota et al., et al., 2018). The native range of this species covers the North-Western Atlantic 79 (Newfoundland and Labrador waters, south-west Greenland shelf to southern Baffin Bay) 80 (Squires, 1990); the North Pacific with south ward border atef the Aleutian Islands and the Sea of 81 Japan (Slizkin, 1982), and the Chukchi Sea westward to the boundary with the East Siberian Sea 82 and eastward to the Beaufort Sea (Slizkin et al., 2007; Sirenko & Vassilenko, 2008). There is only 83 one record of snow crabs on the border between the East Siberian and the Laptev Seas, off the 84 New Siberian Islands (Sokolov et al., 2009). Ch. opilio is an active benthic predator consuming a Formatted: Highlight 85 broad range of invertebrates, and even fish (Tarverdieva, 1981; Chuchukalo et al., et al., 2011; Lovvorn, 2010; Kolts et al., et al., 2013; Zalota, 2017; Zakharov et al., 2018). 86 87 The first record of snow crab opilio in the Barents Sea was in 1996 (Kuzmin et al., 1998) Formatted: Highlight 88 It is possible that the introduction took place approximately between the mid-1980s and 1993 Formatted: Highlight 89 (Alvsvåg et al., 2009; Strelkova, 2016). By the mid-2010s the snow crabs occupied the entire 90 central, eastern, and most of the northern part of the Barents Sea. Uncontrolled snow crab fishery 91 commenced in 2013 in the international fishery enclave between the EEZs of Russia and Norway 92 and the Spitsbergen fishery protection zone (Bakanev et al., 2017; Sundet & Bakanev, 2014). A 93 regulated snow crab fishery in Russia's EEZ of the Barents Sea began in 2016 (Bakanev et al., 94 2016). 95 The snow crab population grew in the Barents Sea and expanded towards the Kara Sea. 96 The first crabs were found on the boundary of the two seas in 2008 (Strelkova, 2016), then in the 97 north-west of the Kara Sea in 2010 and 2011 (Strelkova, 2016; Zalota et al., 2018). Both adults 98 and larvae were caught in the south-western Kara Sea in 2012 (Zimina, 2014). In less than five 99 years after the initial records, Ch. opilio was observed over the entire western Kara Sea shelf 100 (Zalota et al., 2018). A high abundance of adult snow crabs was recorded in 2013 in the south-101 western Kara Sea, between the Yamal Peninsula and the Kara Gate Strait, which is the entrance 102 from the Barents Sea (Strelkova, 2016). In 2014, several size groups of juveniles were present 103 throughout the western shelf and the fjords of the eastern Novaya Zemlya Archipelago, with the 104 most numerous groups presumably originating from larval settling in 2013 (Zalota et al., 2018).

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2011; Zimina, 2014; Bakanev, 2015; Sokolov et al., et al., 2016; Spiridonov & Zalota, 2017;

It is still uncertain if the Kara snow crab population is fully established and independent of larvae

import and adult migration from the Barents Sea, and how far it can expand eastwards.

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The oceanographic conditions of the Kara Sea are very different from the Barents Sea. The western part is strongly influenced by the water exchange with the Barents Sea and by the advection of fresh water from large Siberian rivers' runoff (*Pavlov & Pfirman*, 1995; *Zatsepin et al.*, 2010a; *Zatsepin et al.*, 2010b; *Zatsepin et al.*, 2015; *Polukhin & Zagretdinova*, 2016). The Kara Sea is covered with ice for most of the year, with extensive fast ice massifs and regular polynya formations (*Gavrilo & Popov*, 2011; *Polukhin & Zagretdinova*, 2016). Beginning from the mid-2000s, the Kara Sea follows a general Arctic trend of delaying sea ice formation in autumn and earlier decay in spring/early summer (*Ashik et al.*, 2014). This coincided with the commencement of *Ch. opilio* invasion from the Barents Sea (*Zalota et al.*, 2018).

In comparison to the Barents Sea, the Kara Sea has a much lower primary productivity (*Vinogradov et al.*, 2000; *Romankevich & Vetrov*, 2001; *Demidov & Mosharov*, 2015; *Demidov et al.*, 2015) and benthic biomass (*Zenkevich*, 1963; *Denisenko et al.*, 2003; *Kulakov et al.*, 2004; *Udalov et al.*, 2016; *Chava et al.*, 2017). Its ecosystem is noticeably affected by climate change and the lengthening of ice-free period (*Ashik et al.*, 2014). Persistent accumulation of organic pollution (*AMAP Assessment*, 2015) and a massive offshore and coastal oil and gas development, and shipping (*Amiragyan*, 2017) will influence the Kara Sea in the nearest future. In the nearest future it will be more and more influenced by several anthropogenic factors. These include the persistent accumulation of organic pollution (*AMAP Assessment*, 2015), massive offshore and coastal oil and gas development, and shipping (*Amiragyan*, 2017). The establishment of a breeding snow crabs population, even if dependent on the Barents Sea stock, may have additional large scale impact on the distinct Kara Sea ecosystem. On the other hand, the snow crabs could potentially grow to commercial sizes and become regulated by offshore fishery, which has never existed in the Kara Sea before. It is therefore critical to study the development of the snow crab population in the Kara Sea to forecast the future of the Siberian Shelf ecosystems, and the options

The Barents Sea snow crab population is spatially and temporally monitored in a standardized way (*Jørgensen et al.*, 2015; Strelkova, 2016). The Barents Sea snow crab population is well monitored with comparable gear because of regular ecosystem survey of the IMR and PINRO (*Jørgensen et al.*, 2015). Due to its limited fishery resources, the Kara Sea is less visited and surveyed using standard fishery trawls, which makes it very difficult to obtain a representative samples of adult snow crabs and to monitoring their abundance (*Zimina et al.*,

for resource management and biodiversity conservation.

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2015; Sokolov et al., 2016). Since 2007, smaller scientific gear, such as Sigsbee trawls, has been used in regular expeditions of the Shirshov Institute of Oceanology (SIO) to the Kara Sea. It provides a good representation of juvenile groups, but most likely underestimates large crabs (Zalota et al., 2018). Therefore, to study the population of snow crabs in the Kara and potentially in other Siberian seas in the long term,—we have to be able to use a combination of gears and exploit the methodological opportunities which present themselves.—we need to learn how to combine the data supplied by different gear to draw meaningful conclusions and obtain data any time there is an opportunity

In the summer season of 2016 we employed three methods to study snow crab size composition and abundance: The authors from SIO RAS collected crabs using a Sigsbee trawl, along with video transects; and the MMBI affiliated author used a large Campelen-type bottom trawl similar to those used in the IMR—PINRO surveys in the Barents Sea. The video survey, conducted by SIO RAS, is less invasive, less costly and labour intensive method of rapid assessment of density and size structure of crabs' settlements. However, the video data lacks important information, such as differences in size composition due to sex dimorphism. Therefore, it is important to identify the information that can be safely combined.

The purpose of the present paper is to compare and understand the differences in the results obtained by different gear to study an ongoing invasion of an alien crab in the remote Kara Sea.-the data on population composition and abundance of snow crab in the Kara Sea obtained by these The data from three types of sampling gear is analysed to obtain the size and sex composition, and the density of snow crab settlements in the Kara Seamethods in 2016. By identifying specifics and merging the results obtained by these different gearsgear we also aim to assess the progress of the *Ch. opilio* invasion in the Kara Sea, and to compare it with earlier stages (2008 – 2014) described by *Zimina* (2014), *Strelkova* (2016) and *Zalota et al.* (2018).

# MATERIAL AND METHODS

The crabs *Chionoecetes opilio* were studied using three sampling methods. Deduring the cruise of on the Research Vessel *Dalniye Zelentsy* (MMBI) and the RV *Akademik Mstislav Keldysh* (SIO-RAS) in August-September 2016 (for abbreviations see authors' affiliation). On the RV *Dalniye Zelentsy* MMBI samples were collected using a Campelen-type bottom trawl with a 20 m horizontal by an 8-10 m vertical opening, equipped with a double net; the outer net with

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135 mm mesh and lower insertion of the net with 12 mm mesh. <u>TOn</u> the <u>RV Akademik Mstislav KeldyshSIO</u> the <u>material samples</u> wereas collected using a Sigsbee trawl with a steel frame of 2 m breadth and 35 cm height. The trawl was equipped with a double net; the outer net had 45 mm mesh and inner net had 4 mm mesh.

A video transect was filmed by the team of engineering and technical research iIn combination with some four trawlings by SIO (Fig. 1) RAS, a video transect has been filmed by the team of engineering and technical research. This was done using an uninhabited, towed, submerged, inert vehicle (UTSI) Video Module developed and produced by the efforts of technical and biological specialists of the SIO RAS (Pronin, 2017). UTSI Video Module is equipped with a control and data transmission system that through which allows you to receive information is received and to-control commands are transfered control commands between the vessel and the towed entity via an optical cable in real time. UTSI Video Module has a navigation system, power supply, three video cameras (one of them is of high resolution, set up to carry out planimetric surveys), six floodlight projectors and two laser scale indicators, with a known distance between them (60 cm). If necessary, it is possible to install additional equipment such as a hydrophysical probe, a submersible gamma spectrometer, etc. The use of Our use of the UTSI Video Module allowed us to obtain geo-referenced (including depth), spatially oriented and scaled images of the bottom with organisms (Fig. 7 A,B,D)(Poyarkov et al., 2017; Pronin, 2017).

The team on the RV *Dalniye Zelentsy* has-collected crabs from 53 stations (further referred to as MMBI samples) on the west side of the Yamal Peninsula in the Kara Sea (Fig. 1, circles.). The RV *Akademik Mstislav Keldysh* collected trawl samples (further referred as SIO samples) in the vicinity of four Novaya Zemlya Archipelago bays and two in the area of the Kara Gates Strait (Fig. 1, diamonds). The video transects (further referred as video samples) have beenwere done prior to trawling aton four of these stations (Fig. 1, stars in the diamonds), and in the vicinity of Tsivolka Bay without a trawling sample (Fig. 1, black star). The trawling stations of SIO closely followed the rout of the video transects and can therefore be directly compared. Both, video and trawling sampling was done in the vicinity of Blagopoluchiya, Haug, and Abrosimov bays, as well as in the southern region of the Kara Gates Strait (further referred to as Gates 2) (Fig. 1).

All crabs caught in trawls <u>have been were</u> sexed, based on visual characteristics, and measured (carapace width, CW) using <u>callipers</u> to the nearest millimet<u>ere</u> on board the vessels. <u>In</u>

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this paper crabs with CW less than 11 mm, whose sex cannot be easily visually identified are referred to as the "juveniles". The videos have beenwere viewed using the Media Player Classic - Home Cinema program in full screen mode. Manually, by screenshots, the videos have beenwere divided into still frames according to the changes in the bottom features to account for varying speed of towing, changing depth, and magnification. The height and width of the frame, the distance between two laser points and the carapace width (CW) of crabs present on the image have beenwere measured using a ruler. All frame measurements were converted to the actual dimensions considering that the distance between the laser points on the bottom was 60 cm (Fig. 7 A.B.D).

No native crabs, *Hyas araneus*, were spotted in these videos. However, in the videos taken in other years (not discussed in this work) the native crabs could be successfully differentiated from the snow crabs' opilio in the vicinity of the Kara Gate Strait (A. Zalota unpublished data). Each video frame was visually inspected by one person (A. Zalota) and only frames with acceptable visibility deemed tolerable by the viewer were used. This has been done to minimize the errors related to different interpretations by various observers, which are unavoidable in such a subjective analysis. As this is our first attempt to record and analyze such videos, the quality was not always of the same standard and a large proportion of the data could not be used. Not all measured crabs were used in the density calculations, since some frames had to be cut around the edges to standardize the method.

Most of statistical calculations and analyses have been were performed using RStudio (RStudio Team, 2016; R Core Team, 2018). Size structure of collected crabs has been was analyzed using mixture model analysis in PAST software (Hammer, 2013). The best fit models have been were selected using the Akaike (Akaike, 1974) and log likelihood criteria. To analyze possible trends of crabs' size distribution in space, we looked at the correlation between the depth of sampling stations and different statistical parameters of the CW. Correlations have been were calculated using Microsoft Excel package.

RESULTS

Overall, the data has been collected from 64 sample stations. MMBI trawled at 53 stations and caught 662 crabs; SIO RAS caught 857 from 6 trawling stations and 884 crabs were caught

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on camera at the 5 stations where *Video Module* was employed (Fig.1). In total 2402 crabs were measured, which includes 1520 crabs caught <u>inby</u> trawls.

# Size composition revealed by different sampling methods

Different methods of collection yielded diverse size distribution of crabs. Although the size composition of adult male and female snow crabs usually differs, we discuss their aggregate composition in order to compare the data from trawling data to with the video data, for which no sex differentiation is possible. The carapace width (CW) of crabs caught during SIO trawling ranged from 4 to 117 mm (Fig. 2 A). Mixture analysis of CWs identified 9 distinct size groups from the bulk of SIO crabs (7 groups from the analysis and 2 were added manually to decrease noise during the analysis) (Table 1). The majority of crabs were of small size, with the CW mode CW at 14 mm (while the mean was 16 mm) and another abundant group at CW 10 mm.

The Mmixture analysis of crabs caught by MMBI trawling has-resulted in only 5 size groups (1 of which has beenwas added manually) (Table 1). Overall, crabs caught by this method were of larger size, with the minimum carapace width of 22 mm and the maximum of 120 mm.

AThe large portion of crabs was within the 52 mm size group of 52 mm (mode at 52, mean wasof 57 mm) (Fig. 2 B). The analysis has-merged smaller crabs into one group with large standard deviation (36±7 mm). However, the mixture analysis identified more distinct large size groups (over 52 mm) in MMBI samples, than from SIO trawling and video sampling.

The smallest crab identified on the video had 7 mm CW and the largest 127 mm. Mixture analysis yielded 4 size groups for this method (Table 1). Most frequently crabs were within two broad size groups with CW 15±3 mm and 46±11 mm (Fig. 2 C). The mode CW was 15 mm, however for this method the mean CW differed at 32 mm.

The trawling stations of SIO RAS closely followed the rout of video samples and we can therefore compare them directly (Fig. 4 A, B). Both, video and trawling sampling was done in the vicinity of Blagopoluchiya, Haug, and Abrosimovo bays, as well as in the southern region of the Kara Gates Strait (further referred to as Kara Gates 2) (Fig. 1).

The boxes in Figure 2 (<u>interquartile range containing</u> 50 % of the data, from the first to third quartile) of Blagopoluchiya Bay are very similar for both methods, and include CW below 20 mm. The mode, median and means for the trawling and video samplings in Blagopoluchiya Bay are also very similar (<u>Table 2all three at 14 mm for trawling; and 15 mm mode/median and</u>

17 mm mean for video). However, the maximum size observed by in video (50 mm) was larger than in the Sigsbee trawling sample (38 mm), while the minimum was very similar (7 and 8 mm respectively). Both methods yielded large number of crabs: 735 by trawling and 388 caught on video. The SIO trawling sample revealed more distinct and sharp size groups than the video sample (Fig. 3). However, the The mixture analysis has identified only one more distinct additional size group in the SIO trawling than in the Video sample (Table 1), but the distinction and precision of these size groups are much more apparent in the SIO sample even to the naked eye (Fig. 3).

The two methods <a href="https://mail.org/har-10.2">have shownhad</a> similar results for the south of the Kara Gates Strait (Kara-Gates 2, Fig. 4; Table 2). The central 50% of crabs had CW between 43 and 57 mm (47 mm mode and median, and 48 mm mean for trawling; and 43, 48, and 51 mm for video respectively). Trawl sampling collected 20 crabs, and we observed 186 crabs on the video. While the minimum CW of crabs from trawling and video were very similar, 27 and 22 mm respectively, the video detected much larger crabs (127 mm) than the trawling (71 mm). However, we caught large crabs (up to 117 mm, 50% between 24 and 45 mm; mode, median, mean at 25, 35, 39 mm; for 27 crabs) while trawling in the nearby location (Kara-Gates 1, 2, Figs. 1, 4, Table 2).

We detected a very low number of crabs in Haug Bay <u>by</u> both <u>methods</u> <u>trawling and video</u> (4 and 7 respectively). However, their sizes were very different.: <u>In</u> trawl sample all crabs were 15-16 mm; <u>inon</u> the video <u>sample</u> the size ranged from 27 to 50 mm, while the majority <del>(mode, median and mean)</del> was within 42-47 mm.

The most distinct difference was observed in Abrosimov Bay (Fig. 4 A, B, Table 2). A large proportion of Tthe trawl catch brought a large proportion of were smaller crabs (minimum and mode at 4 mm CW), and median 16 mm, mean 22 mm; the central half of crabs 50% of crabs were between 4 and 32 mm). Owhile on the video we could not observe only larger such small crabs (minimum 9 mm), and the central half of the crabs were much larger, between 34 and 48 mm CW, (mode, median, mean: 48, 43, 40 mm respectively). The maximum size of the largest crabs in both methods were as very similar (50 mm trawling, 58 video). We measured twice as many crabs (88) on the video than infrom the trawling sample (43).

Sedov and Tsivolka Bays were sampled using different methods and cannot be compared directly. Trawling sample in Sedov Bay brought 28 crabs with 45 mm maximum and 10 mm

minimum CW. The central half of crabs were within 14-21 mm CW range; with mode, median and mean at 25, 16 and 19 respectively. On the Tsivolka Bay video we identified 215 crabs from 9 to 58 mm CW. The central half of the crabs fell between 34 and 52 mm CW; mode, median and mean are 50, 46 and 41 respectively.

At the 53 MMBI trawling stations the minimum CW ranged from 22 to 72, and the maximum from 39 to 120. The modes and means ranged from 32 to 94 and 34 to 75 respectively. Most often (mode), the minimum CW was 47 mm, maximum was 57 mm; mode and mean were 52 mm. Two to sixty one crabs were caught at the MMBI sampling stations, most commonly 3 crabs per station, but 12 crabs on average.

To analyze possible trends of crabs' size distribution in space, we looked at the correlation between the depth of sampling stations and different statistical parameters of the CW (Table 2). For tAt the 53 MMBI trawling stations, the maximum, mode and mean CW sizes only weakly correlated with the depth (Table 3). In both SIO trawling and video samples the maximum CW sizes had strong correlation with the depth, although the sample size was very small.

When we mapped the maximum CW size for each station, we observed a trend in the MMBI samples, where along the Yamal Peninsula (Fig. 5). Larger crabs tend to be found in the south and northwards along the Yamal Peninsula,  $\frac{1}{2}$  Teowards the centere of the western Kara Sea the maximum CW of the crabs decrease (Fig. 5). Maximum MMBI CW sizes weakly correlated with the mode and mean CW sizes of those stations (maximum to mode CW: p = 0.5,  $R^2 = 0.3$ ; maximum to mean CW p = 0.9,  $R^2 = 0.4$ ). Similar mapping of ovigerous female findings did not show any observable trends.

# Sex ratio and sex related differences in size composition

Trawling allowed identifying and comparing crabs of different sexes. The male to female ratios were strikingly different between SIO RAS (bays of the Novaya Zemlya Archipelago) and MMBI (west of the Yamal Peninsula) (Fig. 1): 0.8 and 3.8 respectively. There were only 8 ovigerous and 366 non ovigerous females in the SIO RAS samples (ratio 0.02), while there were 72 ovigerous and only 67 non ovigerous females in the MMBI samples (ratio 1.07).

The central half of the CW size distribution of crabs differed between samples for all sexes, except for ovigerous females (Fig. 6). Ovigerous females were within a narrow size range

of 44 and 58 mm (mode, median, mean at 55, 54, 52 mm) in the SIO RAS samples, and between 42 and 72 in the MMBI samples (mode and mean of 62 mm, median 61 mm, the central 50% CW ranged from 58 to 66Table 4).

The non ovigerous females from SIO RAS trawling samples ranged from 11 to 47 mm mm(central half between 14-16 mm; mode, median and mean CW of 14, 15 and 17 mm respectively). The MMBI non ovigerous females were larger and ranged from 22 to 63 mm mm.(central half between 33-52 mm; with mode, media and mean CW of 48, 45 and 42 mm).

Overall, male sizes also differed between these two sampling methods and area of collection: The CW of 293 crabs caught by SIO Sigsbee trawl RAS ranged from 11 to 117 mm. (mid 50% within 14-16 mm; mode, median and mean of 14, 15, and 18 mm); and the CW of the 523 crabs caught by MMBI large trawl ranged from 23 to 120 mm-(mid 50% within 51-66 mm; mode, median and mean of 52, 55, and 59 mm). The Our use of the small mesh in the Sigsbee trawl of SIO RAS allowed us to collect 190 small "juvenile" crabs (less than 11 mm). In this paper we apply this term to all crabs with CW less than 11 mm, whose sex cannot be easily identified.

#### **Abundance estimation**

 Population density of crabs was calculated from the video transects (Table 53). In these videos no native *Hyas araneus* crabs were spotted. However, in videos made in different years (not discussed in this work) the native crabs could be successfully differentiated from the snow crabs opilio in the vicinity of the Kara Gate Strait. Each video frame was visually inspected by A. Zalota and only frames with tolerable visibility were used. As this is our first attempt to record and analyze such videos, the quality was not always of the same standard and a large proportion of the data could not be used. Not all measured crabs were used in density calculations, since some frames had to be cut around the edges to standardize the method. Overall 3132 frames were analyzed, resulting in 3776 m<sup>2</sup> of bottom inspected.

The maximum density was observed near the Kara Gate Strait and in Blagopoluchiya Bay (0.87 and 0.55 crabs/m<sup>2</sup> respectively). In the other bays (located between the Kara Gate Strait and Blagopoluchiya Bay) the population density of crabs was several times or one-an order of magnitude lower, reaching the minimum value of 0.01 crabs/m<sup>2</sup> in Haug Bay (Table 53).

# DISCUSSION

# Advantages and disadvantages of the applied methods to study <u>invasive the</u> snow crab population in the Kara Sea

The three methods discussed here revealed different aspects of the *Chionoecetes opilio* population size structure in the Kara Sea. The Sigsbee trawl used by SIO RAS has small mesh and catches crabs as small as 4 mm CW, which is the size of recently settled crabs (*Conan et al.*, 1996). However, it also has a small opening, and some of large agile crabs evadescape it. The video recording of the same area shows that large crabs are present, although not always caught. The large bottom trawl is able to catch large crabs (22-120 mm CW), but does not retain younger crabs, due to its large mesh (22-120 mm CW). We do however know that at least at some of the MMBI stations juvenile crabs were present. In some cases a similar to the SIO RAS Sigsbee trawl has been was used, but the data was not dully recorded, and is thus omitted from the results. The combined use of thisese trawling gearsgear could provide a full scope datapicture of the existing size groups in a population.

The On the video sampling allows us to it is easy to observe larger crabs, although some smaller crabs can also be spotted (up to 7 mm CW) (Fig 7 A). The *Video Module* floats over the bottom with very little impact. Due to the muddy sediments in the studied area, every sudden movement of large agile organisms (crabs, fish) creates a notable cloud and could easily be spotted on the video. In all of the recorded footage, there were very few cases of such clouds: in most of them it was a fish, and sometimes crabs would run forward, and stop, therefore still recorded by us (Fig 7 C). It is safe to say that larger crabs (CW 30 mm and above) are quantitatively recorded on the video. However, crabs smaller than approximately 30 mm are probably substantially underestimated. Snow crabs are known to borrow in the sediments, especially in younger stages (*Conan et al.*, 1996; *Dionne et al.*, 2003). In some cases with good visibility, an outline of submerged crabs could be seen on the surface of the muddy sediments (Fig 7 B). Although, it is possible that a few crabs were not counted due to low visibility and deep burrowing. Therefore, the crab densities calculated from the video footage can be largely underestimated.

The highest density of crabs recorded in 2016 was in Blagopoluchiya Bay  $(0.87 \text{ crabs/m}^2)$ . The crabs were very small in both the Sigsbee and the video samples (majority of

crabs with CW below 20 mm). Even though the mixture analysis has identified two distinct small sized groups (8-9 mm and 14-15 mm) forer both sampling methods, there was a much sharper difference between the groups, and the larger groups were much more distinct in the trawling samples (Fig. 3 A,B, Table 1). Such differences could be due to possible errors in size measurements of filmed crabs. In case of the trawling samples, the CW was measured directly, using callipers. In the video samples, the CW was measured by a ruler, which has lower precision. In addition, the measurements were recalculated based on the distance between the two laser points that were also measured by a ruler. The crabs were not always in absolutely plain position towards the camera, and the visibility often did not permit to see the edges of carapace clearly. Therefore, there could be some additional noise in CW measurements is a lot of guessing involved in attaining the CW measurements frofrom them video footage in comparison to the direct measurements by calipers of live organisms.

However, the most important error in the identification of size groups using video samples was due to sexual dimorphism. The size groups (instars) of Ch. hionoccetes opilio have been are extensively described in the literature well studied and the young crabs (pre puberty molting <20-30 mm) seem to have similar size groups across their range of habitat (*Ito*, 1970; *Ogata*, 1973; Kon, 1980; Sainte-Marie et al., 1995; Ernst et al., 2012). These instars are in accordance with those observed for young crabs in the Kara Sea in 2014 (Zalota et al., 2018) and in 2016 (present study, Table 1). After puberty molting (which was shown to be at 37-40 mm for males and 17 mm for females, in the Gulf of St. Lawrence) crabs' growth rate and possible skipping of moults in females is strongly affected by temperature (Sainte-Marie et al., 1995; Alunno-Bruscia & Sainte-Marie, 1998; Dawe et al., 2012), which is less than 0 and -1°C in most areas of the Kara shelf (Polukhin & Zagretdinova, 2016; see also Zalota et al., 2018). Further growth and survival success of larger crabs may also be affected by low food availability for benthic predators in the Kara Sea (Zenkevich, 1963; Kulakov et al., 2004). As they age further, they molt approximately once a year, or even rarer until they reach their terminal molt (males at CW (postmoult) as small as 40 mm up to 150 mm; females 30-95 mm) (Ito, 1970; Robichaud et al., 1989; Comeau et al., 1998; Sainte-Marie & Hazel, 1992; Sainte-Marie et al., 1995; Alunno-Bruscia & Sainte-Marie, 1998).

Taking into account these errors, caused by aggregating males and females in video samples, it is not surprising that crabs larger than 20 mm blur into one large size group in the

video obtained data, while crabs caught by the large MMBI trawl and measured more accurately can be separated into at least 4 size groups (Table 1, Fig. 2 B, C). Even finer size structure in the MMBI samples can be seen if crabs over 20 mm are separated according to sex (Table 1). Males have 7 distinct size groups over 20 mm CW, whereas, females' groups are more blurred. This can be due to the differences in molting and growth rates. Since video data cannot provide information on sexual dimorphism, the work done to study exact differences in the size structure of immature and mature crabs in the Kara Sea is not presented in this paper. Nevertheless, the obtained data still permits to identify generalized size groups and to approximate their relative quantities.

### Development of snow crabs' invasion and possible role in Blagopoluchiva Bay

Blagopoluchiya Bay appears to have very different size structure compared to all other samplessampled areas. Most of the crabs, both caught in the trawl and in the video footage, were less than 20 mm, and form 2 high frequency groups at around 105 and 150 mm CW (Figs. 3 A,B, 4; Table 1). These size groups correspond to the age of less than 2 years. In the Gulf of St. Lawrence it takes 16 to 17 months for crabs to achieve CW 10 mm, and another 16 months to achieve size 20 mm through multiple molting events (*Ogata*, 1973; *Sainte-Marie et al.*, 1995). Therefore, the majority of these crabs could not have settled much earlier than 2014. That year we caught crabs no bigger than 5 mm in the vicinity of that bay (station 51 in *Zalota et al.*, 2018). Indeed, that was the first year when the crabs have been observed across the entire western Kara Sea and in most cases they were young, with high abundance of just settled crabs (*Zalota et al.*, 2018).

There were a few larger crabs in Blagopoluchiya Bay that were caught on camera. Their sizes are not big enough to assume that they ir activelye migratedion from other areas. This suggests that there were earlier successful settlings of crabs, but the proportion of larger crabs is almost negligible (Fig. 3 A, B). The density of the young crabs in the bay is very high (0.87 crabs per m²) and in some cases we observed up to 8 crabs in one video frame (approximately 0.5 m²). Such high densities of young crabs suggest that the recently particular a combination of favourable oceanographic and sea ice conditions in the area facilitated a massive settling and

<u>survival</u> and rapid increase of the juveniles snow crabs numbers. The larvae that settled in Blagopoluchiya Bay were likely transported by the Eastern Novaya Zemlya current.

This current originates from the Barents Sea water, entering the Kara Sea off the northern coast of the Novaya Zemlya and is directed to the south-west along the eastern coast of this archipelago (*Pavlov & Pfirman*, 1995). Previously, the bays of the Novaya Zemlya, especially in the north such as Blagopoluchiya Bay, have been blocked by the ice longer than most of the western Kara Sea (*AARI*, 2007-2014). Although a narrow Northern Novaya Zemlya Polynya occurred from time to time (*Gavrilo & Popov*, 2011). Since 2011, changing sea conditions of the 2000 – 2010s manifested in an abrupt sea ice decrease in June (*NOAA*, *Snow and Ice*, 1979-2018). Although, there was more ice in the spring of 2014 (in comparison to 2011), the sea ice cover along the Novaya Zemlya Archipelago, was the first to retreat and an extensive polynya was formed (*AARI*, 2007-2014). Early sea ice decay could have facilitated seasonal development of phyto- and zooplankton, and hence favourable conditions for feeding and successful settling of crab larvae.

This When the first snow crabs were settling, they experienced small predator pressure in the Kara Sea benthos, took place in the practical absence of benthic predators. Adult snow crabs that are highly cannibalistic were not yet present, and the native crab species, *Hyas araneus*, are very rare in that area (*Anisimova et al.*, 2007; authors' observations). In addition, predatory demersal fishes have substantially lower diversity and abundance in the Kara than in the Barents Sea (*Dolgov at al.*, 2009, 2014; *Dolgov & Benzik*, 2016).

<u>Initially</u> The snow crabs could have settled in the southern bays and in the central part of the Kara Sea (which in addition to larvae transportation adults could have also reached it by active migration) before they reached the northern bays of the Novaya Zemlya Archipelago. Hence, we can observe such difference in the population size structure of Blagopoluchiya Bay (narrow range of size groups) in comparison to the rest of studied southern areas (all size groups are present).

## Indication of formation of spatial population structure

Overall there seems to be a pattern in the snow crabs' size distribution across the western Kara Sea. In the southern Kara Sea these patterns are not related to depth since there is no strong

correlation between the CW and the depth across allAll MMBI stations (large bottom trawl), do not have strong correlation between the CW and the depth. These stations are positioned on a broad, slightly sloping shallow shelf (46 to 195 m). However there is a visually observable trend of maximum sizes prevailing in the Kara Gates Strait and northwards along the Yamal Peninsula (Fig. 5). This closely resembles prevailing current path of the Barents Sea waters, known as the Yamal Current (Pavlov & Pfirman, 1995; Zatsepin et al., 2010a). The area along the Yamal Peninsula and the Novaya Zemlya Archipelago has higher benthic biomass rates than in the centerre of the western Kara Sea (Antipova & Semenov, 1989; Denisenko et al., 2003; Kulakov et al., 2004; Kozlovskiy et al., 2011). The decrease of the maximum CW from the Yamal Peninsula towards the centerre of the western Kara Sea could be due to lower food availability further away from the Barents Sea influence in the centre, and thus the crabs have insufficient nutrition to achieve larger sizes. However, this hypothesis needs further confirmation.

There could also be behavioural separation, where smaller sized crabs are forced to move to athe territory with less rich in food territory to escape cannibalism, which is very common among this species (*Conan et al.*, 1996; *Comeau et al.*, 1998). No such trends can be observed along the Novaya Zemlya Archipelago, probably due to low sampling effort. There are reports of difference in the habitat preferences of different sized snow crabs in their native habitat areas (*Comeau et al.*, 1998; *Ernst et al.*, 2012). The bays of the Novaya Zemlya have the potential to act as a nursery for smaller, more vulnerable specimens, as it has been observed in the Gulf of St. Laurence (*Comeau et al.*, 1998). Whether this separation exists or will ever exist in the Kara Sea is hard to say at this point. However, the vicinity of deep trough along the Novaya Zemlya Archipelago could attract larger crabs and lead to size based related migration out of the bays.

The recruitment of crabs in the Kara Sea at the early stages of the invasion might be mostly due to the inflow of the larvae from the Barents Sea (*Zalota et al.*, 2018). Here we present findings of substantial number of ovigerous females, most of which have been found along the Yamal Peninsula (with no apparent spatial or depth distribution patterns), and none in the bays. I with the present data it is hard to say whether this was due to sampling gear limitation to catch representative sample of larger crabs or a reflection of the real picture. All ovigerous females had CW larger than 40 mm (Fig. 6). This corresponds to the size of female's terminal (sexual maturity) molt reported in the literature (starting from 35 mm) (*Sainte-Marie et al.*, 1995; *Alunno-Bruscia & Sainte-Marie*, 1998). Crabs of these sizes had a low catchment rate in the SIO

trawling samples along the Novaya Zemlya Archipelago. In most cases the video samples suggest that the crabs with CW larger than 40 mm prevail in the vicinity of most sampled bays (Fig. 4). Therefore, it is likely that there are reproducing crabs in most sampled areas. Therefore I it is safe to say, that at present the snow crab opilio has a reproducing population in the Kara Sea.

The spatial and functional structure of the snow crab population in the Kara Sea is still in process of formation. The data of 2016 indicate that this process may lead to a quite complex system, which is based on local recruitment, transport of larvae from the Barents Sea and across the western Kara shelf, formation of nursery grounds, and an active migration of adults and their concentration in particular shelf areas with appropriate feeding conditions. This system on the other hand can't be static as it is influenced by changing advection of the Barents Sea water and its interaction with the water of river discharge origin (*Zatsepin et al.*, 2010b), sea ice regime, trophic conditions and predation pressure on juvenile crabs.

521 CONCLUSION

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The present study compares and combines the results obtained using three different sampling gears gear to assess the size composition and density of the snow crab population. Smaller (Sigsbee trawl allows chatching of small crabs, even those that are just settled. A large commercial type trawl catches large agile crabs and results in a larger number of ovigerous females. survey, Vvideo transects probably underectimates smaller crabs, but gives a rapid and accurate estimate of larger crabs' densities. This method is helpful to monitore the spatial progrees of the crabs' invasion and the appearance of commercial sized crabs. Trawling is necessary to study reproductive biology of crabs in new conditions and to carefully identify size structure of the population., and a survey performed using a large commercial type trawl). These methods prove to be complementary in assessing a complex structure of the developing snow erab population in the Kara Sea. Optimally they have to be used alongside. The data of 2016 has finally proven that the Kara Sea population has attained some complexity with all age groups present and is reproducing, although presumably still strongly influenced by the larval transport from the Barents Sea. We observed initial nursery areas in the bays of the eastern coast of the Novaya Zemlya Archipelago. A number of commercial sized crabs can be observed and areas with large adult concentrations near the Kara Gate Strait and along the western coast of the

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Yamal Peninsula with higher food availability than the rest of the Kara Sea. Only further studies of the invading Chionoecetes opilio population can show how persistent these features are. **ACKNOWLEDGEMENTS** We would like to thank the Captains, the crews and the science teams and their leaders that participated during the RV Akademik Mstislav Keldysh SIO RAS and RV Dalniye Zelentsy MMBI KSC RAS cruises to the Kara Sea in 2016. We are particularly grateful to the team of engineering and technical research of SIO RAS for creating and using an uninhabited, towed, submerged, inert vehicle (UTSI) Video Module to film the footage during the SIO RAS expedition. REFERENCES AARI, 2007-2014, General maps of sea ice cover. St. Petersburg: Arctic and Antarctic Research Institute (AARI), http://www.aari.ru/odata/\_d0004.php?m=Kar&lang=0&mod=0&yy=2014 (Accessed 25.11.2018). AARI, 2009, Winter Kara Sea Atlas, http://www.aari.ru/resources/a0013\_17/kara/Atlas\_Kara\_Sea\_Winter/text/rejim.htm (Accessed 25.11.2018). Akaike H. 1974. A new look at the statistical model identification. IEEE Transactions on Automatic Control 19:716-723. Alunno-Bruscia, M., Sainte-Marie, B. 1998. Abdomen allometry, ovary development, and growth of female snow crab, Chionoecetes opilio (Brachyura, Majidae), in the northwestern Gulf of St. Lawrence. Canadian Journal of Fisheries and Aquatic Sciences. 55(2): 459-477. Alvsvåg, J., Agnalt, A. & Jørstad, K. 2009. Evidence for a permanent establishment of the snow crab (Chionoecetes opilio) in the Barents Sea. Biological Invasions, 11: 587-595. AMAP Assessment, 2015. Temporal Trends in Persistent Organic Pollutants in the Arctic. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway. vi+71pp Amiragyan, A. 2017. Development of oil and gas resources of the Russian Arctic shelf: problems

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