Title	Diets and body condition of polar cod (Boreogadus saida) in the northern Bering Sea and Chukchi Sea					
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Abstract To understand trophic responses of polar cod *Boreogadus saida* (a key species in Arctic food webs) to changes in zooplankton and benthic invertebrate communities (prey), we compared its stomach contents and body condition between three regions with different environments: the northern Bering Sea (NB), southern Chukchi Sea (SC), and central Chukchi Sea (CC). Polar cod were sampled using a bottom trawl and their potential prey species in the environment were sampled using a plankton net and a surface sediment sampler. Polar cod fed mainly on appendicularians in the NB and SC where copepods were the most abundant in the environment, while they fed on copepods, euphausiids, and gammariids in the CC where barnacle larvae were the most abundant species in plankton samples on average. The stomach fullness index of polar cod was higher in the NB and SC than CC, while their body condition index did not differ between these regions. The lower lipid content of appendicularians compared to other prey species is the most plausible explanation for this inconsistency.

**Keywords:** gelatinous zooplankton, lipid content, prey availability, regional differences, stomach fullness

38 Introduction

Arctic marine communities are experiencing changes in the timing of formation and retreat of sea ice and increases in seawater temperatures (Leu et al. 2011, Grebmeier 2012). Polar cod (*Boreogadus saida*) is an abundant epipelagic fish found throughout the Arctic Ocean (Ponomarenko 1968; Bradstreet et al. 1986; Cohen et al. 1990), which feeds on pelagic zooplankton (copepods, hyperiids) and benthic crustacean (gammariids and mysids; Craig et al. 1982; Rand et al. 2013), and is an important food for other fish, marine mammals and seabirds (Welch et al. 1992). Thus this species is a key component in Arctic marine food webs (e.g., Hop and Gjøsæter 2013). To understand how changes in Arctic marine food webs may impact polar cod, a study of their trophic responses (changes in diet and energy stores) to differences in zooplankton and benthic invertebrate communities across regions with different environments was conducted.

The diets of polar cod have been studied in the Bering, Chukchi, and Beaufort seas (Lowry and Frost 1981), the northern Bering Sea (Cui et al. 2012), the northeastern

seas (Lowry and Frost 1981), the northern Bering Sea (Cui et al. 2012), the northeastern Chukchi Sea (Coyle et al. 1997), and the western Beaufort Sea (Rand et al. 2013). These studies reported that polar cod opportunistically fed on prey species that were available in a given depth range and region. Thus, regional differences in their stomach contents may reflect prey selection by polar cod and regional variations in prey availability. Further, regional variations in the abundance and quality of diets, i.e., lipid content, as well as marine physical conditions including water temperature, may influence growth rates of polar cod (Hop et al. 1997).

To understand how regional differences in the availability of zooplankton and benthic invertebrates relate to the diet, feeding success and energy stores of polar cod, we examined their stomach contents, stomach fullness and body condition, and the abundance of zooplankton and marine invertebrate as potential prey in the water column and in sediment. We compared these between three regions with different marine environments and communities (Grebmeier et al. 2006, Eisner et al. 2013) during the summer of 2013: the northern Bering Sea (NB), southern Chukchi Sea (SC), and central Chukchi Sea (CC) (Fig. 1).

## **Materials and Methods**

69 Sampling of polar cod

Sampling was conducted aboard the T/S *Oshoro-Maru* (Hokkaido University, Japan) at 12 stations (St; Fig. 1) at depths between 34 and 68 m during July 4–17, 2013. Polar cod were collected during the daytime using an otter bottom trawl (10 mm cod-end mesh size). The net was towed for 15 min over the seafloor at a speed of 3–4 knots. With a given towing speed and warp length, the width and height of the mouth opening were estimated to be 23.6–25.2 m and 4.3 m, respectively, when the net was towed over the seafloor. The depth of the head-rope was monitored by an attached depth sensor and was 3.5–4.3 m above the sea floor. Thus the foot-rope was assumed to be in contact with the seafloor. The area swept by the bottom trawl at each station was computed as the horizontal mouth opening multiplied by the towing distance (based on towing speed and duration). The density of polar cod was calculated as the number of fish per unit survey area. The net might capture some polar cod when it was going down to and up from the seafloor but we could not separate these from fish caught when the net was contacting the seafloor.

Size and stomach contents of polar cod

Two hundred and thirty eight fish samples were frozen at  $-20^{\circ}$ C immediately after collection and stored until analysis. The samples were thawed in flowing water, weighed individually using an electronic balance (0.1 g) and their total length was measured (1 mm). Stomachs were removed and preserved in 10% v/v borax buffered formalin. Stomach contents were weighed using an electronic balance (0.1 mg). To evaluate the current feeding success and energy stores, the stomach fullness index (stomach contents mass / body mass without stomach contents × 100) and the body condition index (body mass without stomach contents / total length<sup>3</sup> × 10<sup>6</sup>) were calculated for each fish.

To examine prey composition, prey items were separated into taxonomic groups using a stereoscopic microscope, weighed (0.1 mg) again, and counted. Stomach contents of all individuals collected at a given station were combined (excluding 27 empty stomachs). The prey composition at each station was summarized by the index of relative importance, IRI  $\% = F_i \times [N_i + W_i]$  (Pinkas et al. 1971), where  $N_i$  is the numerical percentage of prey i,  $F_i$  is the percentage of stomachs containing prey i, and  $W_i$  is the mass percentage of prey i. We categorized taxonomic groups following Rand et al. (2013). Our results indicated that these prey groups accounted for 92% IRI and the remaining zooplankton was characterized as "others".

Zooplankton and benthic invertebrates in the environment

To determine density of potential prey species in the environment, pelagic zooplankton and benthic invertebrates were collected on the same day and at the same place as the bottom-trawl. Pelagic zooplankton was collected with a NORPAC net (mouth diameter 45 cm, mesh size  $335 \mu m$ , flow-meter attached) towed vertically with a speed of 1 m/s

in day-light hours or at night. Since the net was towed from 5 m above the seafloor to the surface, sampling biases attributed to daily vertical migration of zooplankton can be ignored. Samples were preserved in 5% v/v borax buffered formalin. During post-cruise analyses, these zooplankton samples were sorted, counted, and identified to the lowest identifiable taxon, and categorized into the same groups as those used in the diet analysis. Total wet mass of each sample was measured (0.01 g). Density of pelagic zooplankton sampled by the NORPAC net was calculated as the number of individuals or total mass per unit effective area (m²) of water filtered (the filtered volume divided by the towing depth).

Benthic invertebrates were collected using a Smith-McIntyre grab (0.1 m²), with three replicate samples at each station. The sediments were washed using a 1 mm mesh-sieve and prey items were fixed in buffered 10% formalin, and then preserved in 70% ethanol. As potential prey for polar cod, gammariids were counted and weighed. Density for benthic gammariids was calculated as mass per unit area of the grab sampler.

In addition, potential prey species near the seafloor, in particular appendicularians, were observed using a ROV (*Arkas*, Kowa Company, Ltd., Japan) equipped with 150 mm parallel lasers. The ROV was moving 1–2 m above the seafloor with a speed of 0.1–0.2 m/s while recording video images. From these video-images, we scored the abundance of appendicularian houses during every 10-second window as either 0 (absence), 1 (1–2 houses), 2 (3–10<sup>2</sup> houses), or 3 (>10<sup>2</sup> houses) for a total of 300 seconds at each station and defined the score at each station as the maximum score recorded.

Statistics

To examine regional differences in total length, stomach fullness index and body condition index of polar cod, we conducted multiple comparisons using the Steel-Dwass pairwise non-parametric test.

139 Results

140 Size and stomach contents of polar cod

Polar cod were most abundant at St05 (Fig. 1). No fish were collected at St03 and a single fish was collected at St04. Polar cod collected in the NB and the SC were larger than those collected in the CC (Steel-Dwass test, P<0.01, Table 1). The stomach fullness indices were higher in the NB and SC than in the CC, while body condition indices did not differ between the regions (Table 1).

Prey items were found in 211 stomachs out of 238 fish collected. Although the percentage IRI of each prey type varied between the stations (Fig. 3), the average %IRI across stations within the region showed that appendicularians (Fig. 2a) were the most dominant prey found in the polar cod stomachs collected in the NB (47%) and SC (50%), while in the CC appendicularians were completely absent (Fig. 3). In the CC, polar cod fed on a variety of prey including copepods, gammariids, and euphausiids (Fig. 3).

Zooplankton and benthic invertebrates in the environment

In the water column, copepods were abundant in the NB  $(47 \times 10^3/\text{m}^2)$ , the average density across stations within the region), copepods  $(50 \times 10^3/\text{m}^2)$  and barnacle larvae  $(44 \times 10^3/\text{m}^2)$  were abundant in the SC, while barnacle larvae  $(22 \times 10^3/\text{m}^2)$  were more

abundant than copepods  $(12 \times 10^3/\text{m}^2)$  in the CC (Fig. 4a). Appendicularians were less common than other taxa in the NB  $(4 \times 10^3/\text{m}^2)$  and SC  $(10 \times 10^3/\text{m}^2)$ , and were rare in the CC  $(1 \times 10^3/\text{m}^2)$ . The total biomass of zooplankton in the water column varied between stations (Fig. 4b) but the regional average was greatest in the SC  $(54 \text{ g/m}^2)$ , followed by the NB  $(43 \text{ g/m}^2)$  and the CC  $(17 \text{ g/m}^2, \text{ Fig. 4b})$ . The biomass of gammariids in the sediments varied by station and did not show apparent regional differences (Fig. 4c).

The most conspicuous items in ROV video-image were the houses (3–4 cm length) of appendicularians (Fig. 2b). High scores (3) at St06 and St07 (Fig. 4d) indicated that appendicularians were abundant in the SC.

169 Discussion

Diet of polar cod

Given the geographic scope of the study, sampling effort (only 2–5 stations for each of three regions) was rather low, and the variability of the prey consumed (Fig. 3) and that of the zooplankton and benthic invertebrates available in the environment across the stations (Fig. 4) appeared to be high. Nevertheless, some clear patterns emerged. In particular, appendicularians seemed to be a major component of polar cod diets in the northern Bering Sea (NB) and in the southern Chukchi Sea (SC) but were completely absent in the polar cod stomachs in the central Chukchi Sea (CC). Since appendicularians were less abundant than copepods at stations in the NB and SC, the regional variation in the diet of polar cod could not be explained by the composition of zooplankton in the environment. However, the absence of appendicularians in polar cod diets in the CC is consistent with their absence or very low abundance in zooplankton

samples.

The reason why polar cod fed on appendicularians in the NB and SC in spite of high abundances of copepods were unclear. Gelatinous appendicularians contain proportionally less lipid (<0.1%–0.5% in wet weight) than copepods (1.3%–5.7%), gammariids (approximately 18%), and euphausiids (2.2%–10.7%; Nomura and Davis 2005). Thus polar cod in our study did not appear to select prey based on lipid content. The ROV images showed the large appendicularian houses that were easily detected by human eye (Fig. 2a). Appendicularians are also preyed upon by Pacific salmon and Gadiformes, presumably because they move slowly and lack a hard carapace (Purcell et al. 2005). Thus we hypothesize that polar cod feed selectively on appendicularians because their large and conspicuous houses and slow swimming speeds make them easily available as prey.

Stomach fullness and body condition of polar cod

The stomach fullness index of polar cod collected in the NB and SC was greater than in the CC, possibly reflecting the higher biomass of zooplankton in the water column in the SC and NB than CC. Matsuno et al. (2011) also found that the total biomass of zooplankton was higher in the SC than in the CC. Inflow of nutrient-rich water from the Pacific (Eisner et al. 2013) induces higher primary production in the SC than CC, which presumably explains higher zooplankton abundances. This high primary production, seasonal ice cover and shallow water depth may also support a larger biomass of benthic communities in the NB and SC (Grebmeier et al. 2006). In addition to the availability of prey, water temperature might influence foraging activities and hence stomach fullness. The prey consumption rate of walleye pollock (*Gadus chalcogrammus*) is known to

increase with temperature in the laboratory (Kooka et al. 2007). Water temperatures at the seafloor in our study, however, were highest in the SC  $(0.8^{\circ}\text{C} - 1.3^{\circ}\text{C})$ , followed by the CC  $(-1.7^{\circ}\text{C} - -0.8^{\circ}\text{C})$  and then the NB  $(-1.7^{\circ}\text{C} - -1.6^{\circ}\text{C})$ ; indicating that ambient temperature might not explain observed differences in stomach fullness.

The higher stomach fullness in the NB and SC was not associated with higher body condition indices in these regions. This was possibly because polar cod had a lower quality diet consisting of a high proportion of gelatinous appendicularians in the NB and SC. Similarly, Kaga et al. (2013) reported that chum salmon (*Oncorhynchus keta*) had small lipid stores when they fed on gelatinous zooplankton. Another explanation could be that the body condition index reflects the total amount of food eaten during a few weeks or a month before sampling while the stomach fullness index reflects the current food intake. Thus the lower stomach fullness in the CC might not be reflected in body condition. Further study of stable isotope values or fatty acid signatures in the tissue of polar cod can help identify prey species consumed over a longer period and improve our understanding of regional variation in the bioenergetics of polar cod.

## Ecological implications

The reduced sea-ice coverage has been proposed to favor a phytoplankton/zooplankton dominated ecosystems over a sea-ice algae/benthos ecosystem (Grebmeier 2012), while the expansion of warmer Pacific water into the southern Arctic Ocean induced the dominance of warm water copepod species (Matsuno et al. 2011, Questtel et al. 2013). Climate change may also influence the distribution and abundance of gelatinous zooplankton. Kattner et al. (2007) hypothesized that, in the Arctic Ocean, the recent

increases in water temperature and freshwater inflow may result in increased abundances of gelatinous zooplankton. Deibel et al. (2005) also suggested that the abundance and the biomass of appendicularians, which have a short life-cycle, will increase rapidly in Arctic polynyas if the open waters surrounded by sea-ice appear earlier in the season and remain longer. Thus these expected climate-induced changes in pelagic zooplankton and benthic invertebrate communities, either to the gelatinous zooplankton or to warm water living copepods may influence the diet and body condition of polar cod, and hence their recruitment as has been found in Atlantic cod (*Gadus morhua*; Rätz and Lloret 2003).

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## **Ethical Statement**

This work was funded by Green Network of Excellence program (lead by T Kikuchi) of the Ministry of Education, Culture, Sports, Science, and Technology, Japan and by Faculty of Fisheries Sciences, Hokkaido University. The authors declare that we have no conflicts of interest. This article does not contain any studies with human participants performed by any of the authors. This article does not contain any studies including animals that require the permission from Ethics Committee of Hokkaido University. Informed consent was obtained from all individual participants included in the study.

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333	Figure legends
334	
335	Fig. 1 Sampling stations (St) and abundance of polar cod (catch per unit effort as
336	number of fish per km <sup>2</sup> of bottom-trawling net-haul area) in the northern Bering Sea
337	(NB), southern Chukchi Sea (SC) and central Chukchi Sea (CC). No fish were collected
338	at St03.
339	
340	Fig. 2 The body of appendicularians observed in the stomach of apolar cod collected at
341	St02 in the northern Bering Sea (a), and ROV image showing the floating houses of
342	appendicularians at St07 in the southern Chukchi Sea (b)
343	
344	Fig. 3 The percentage composition of prey taxa, shown by the index of relative
345	importance (% IRI), found in the stomachs of polar cod collected in the northern Bering
346	Sea (NB), southern Chukchi Sea (SC) and central Chukchi Sea (CC). Sample sizes
347	(number of stomachs) are shown at the top of bars.
348	
349	Fig. 4 The numerical abundance of each prey taxa (a) and the total biomass of
350	zooplankton (b) in the water column samples collected by NORPAC net tows (4.79-
351	7.61 m <sup>3</sup> water volume), the biomass of benthic gammariids in the sediment samples
352	collected by a Smith-McIntyre grab (0.1 m <sup>2</sup> ) (c), and the score of the approximate
353	numerical abundance of appendicularians (0, absence; 1, 1–2 houses; 2, 3–10 <sup>2</sup> houses; 3,
354	>10 <sup>2</sup> houses) observed by ROV (d) at each station (Fig. 1) in the northern Bering Sea
355	(NB), southern Chukchi Sea (SC) and central Chukchi Sea (CC). The number or mass
356	per unit area (m <sup>2</sup> ) are shown. For (a) and (b) the effective area of the NORPAC net tow

was calculated as the filtered volume divided by the depth of tow and the densities are

presented as the number or mass per unit effective area.

Table 1 Total length, stomach fullness index (proportional stomach content mass to body mass) and body condition index (residual of the size corrected body mass) of Polar cod in the central Bering Sea, southern Chukchi Sea and central Chukchi Sea. Mean±SD, ranges and sample sizes are shown. Regional difference was examined using Steel-Dwass test.

	Northern Bering Sea (NB)	Southern Chukchi Sea (SC)	Central Chukchi Sea (CC)	Steel-Dwass test t-statistic (p-value)		
				NB vs. SC	NB vs. CC	SC vs. CC
Total Langth (mm)	155.9±27.8	140.2±30.4	117.2±25.4	1.9 ( <i>p</i> =0.13)	5.3 ( <i>p</i> <0.01)	5.0 ( <i>p</i> <0.01)
Total Length (mm)	(110.0–230.0, 23)	(87.0–196.0, 78)	(72.0-186.0,130)			
Stomach fullness index	$0.8 \pm 0.4$	$0.9 \pm 0.8$	$0.7 \pm 0.9$	0.8 ( <i>p</i> =0.69)	3.4 ( <i>p</i> <0.01)	3.3 ( <i>p</i> <0.01)
Stomach runness muex	(0.1-1.8, 29)	(0.0-4.5, 78)	(0.0-5.8, 131)			
Dody condition index	$7.1\pm0.3$	$6.9 \pm 0.5$	$6.9 \pm 0.6$	1.6 ( <i>p</i> =0.24) 1.7 ( <i>p</i>	1.7 (0.22)	0.1 (=-0.00)
Body condition index	(6.2–7.7, 23)	(5.6–8.4, 78)	(5.2–8.5, 129)		1.7 ( <i>p</i> =0.22)	0.1 ( <i>p</i> =0.99)

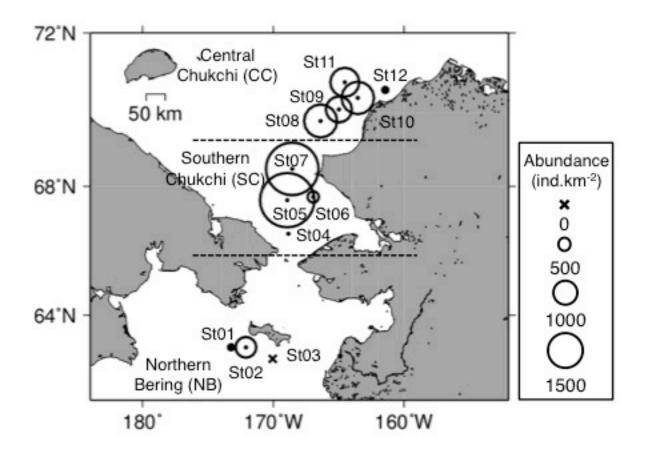


Fig. 1

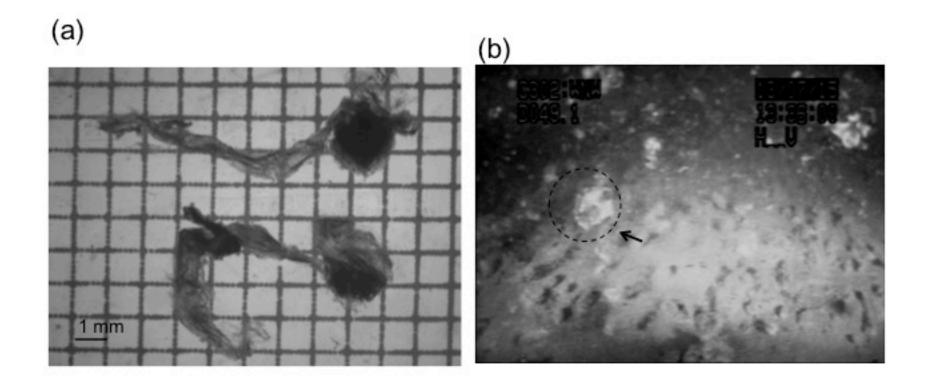


Fig. 2

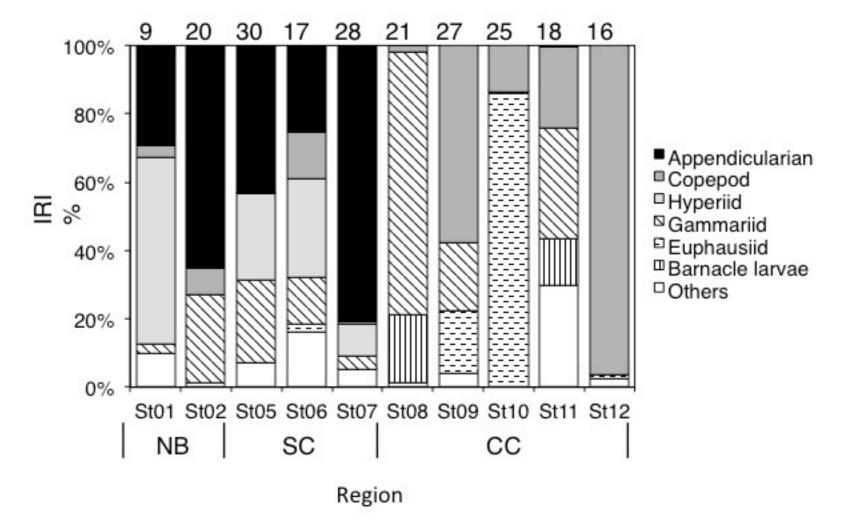
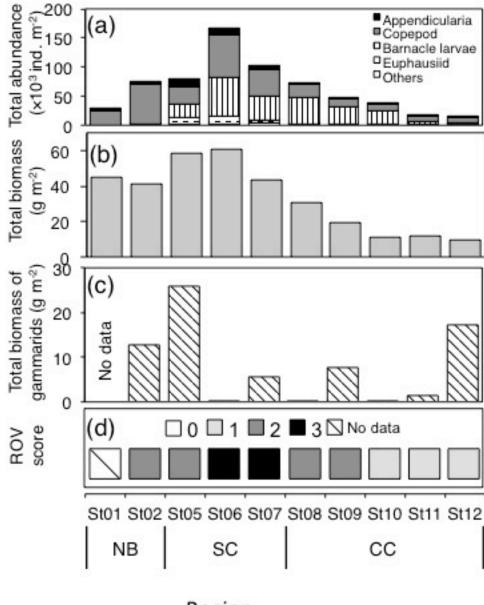


Fig. 3



Region

Fig. 4