Energy budget, growth and exercise as proxies for performance capacity and fitness in Arctic fishes

Dissertation

zur Erlangung des akademischen Grades

– Dr. rer. nat. –

dem Fachbereich 2 Biologie/Chemie

der Universität Bremen

vorgelegt von

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Diplom-Biologin

Bremen, 2019

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TAG DES PROMOTIONSKOLLOQUIUMS: 01.03.2019

Design cover picture: Simon S. Plötz

Diese Arbeit ist inhaltlich identisch zu der beim Prüfungsamt eingereichten Version vom 17.01.2019. Jedoch wurden in der vorliegenden Arbeit geringfügige formelle Änderungen auf den Seiten 53, 73, 121 – 125, 158, lxviii, xli und liii durchgeführt.

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Abbreviations

ANOVA	analysis of variance
AS	absolute aerobic scope
AS_{dig}	absolute aerobic scope of digestion
AS_{ex}	absolute aerobic scope of exercise
A. polyacanthus	*Acanthochromis polyacanthus*, spiny damselfish
ATP	adenosine triphosphate
AWI	Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research
BC	burst count
BC_{max}	maximum burst count
BC_{tot}	total number of bursts
BF	Billefjorden
BL	body length
bpm	beats per minute
BW	body weight
B. saida	*Boreogadus saida*, Polar cod
CA	carbonic anhydrase
$CaCO_3$	calcium carbonate
CAT	constant acceleration test
CF	condition factor
CH_4	methane
CO_2	carbon dioxide
CO_3^{2-}	carbonate ion
C. harengus	*Clupea harengus*, herring
cO_2	oxygen concentration during full oxygen saturation
DIC	dissolved inorganic carbon
dph	days post hatching
D. labrax	*Dicentrarchus labrax*, European sea bass
EPOC	excess post exercise oxygen consumption
E	excretion
E_{max}	maximum swimming efficiency
F	daily food consumption
F_{meal}	food consumption per meal
FCE	food conversion efficiency
FS	Forlandsundet
G	growth
GHG	greenhouse gas
GSI	gonadosomatic index
G. morhua	*Gadus morhua*, Atlantic cod; here: Northeast Arctic cod
H^+	hydrogen ion
HCO_3^-	bicarbonate
HiT	Havbruksstasjonen i Tromsø
HSI	hepatosomatic index
HS	Hinlopenstretet
H_2CO_3	carbonic acid
I	ingested energy
IPCC	Intergovernmental Panel on Climate Change
KF	Kongsfjorden

$KHSO_4$	potassium bisulfate
M	metabolism
MMR	maximum metabolic rate
MMR_{dig}	maximum metabolic rate evoked by digestion
MMR_{ex}	maximum metabolic rate evoked by exercise
MMR_{ex+dig}	maximum metabolic rate evoked by exercise and digestion
$\dot{M}O_2$	oxygen consumption
MS-222	ethyl 3-aminobenzoate methanesulfonate
N_2	nitrogen
NBS	National Bureau of Standards
NEAC	Northeast Arctic cod
NH_4^+	ammonium
NMDS	non-metric multidimensional scaling
NO_2^-	nitrogen dioxide
NO_3^-	nitrate
N_2O	nitrous oxide
OA	ocean acidification
OAW	ocean acidification and warming
OCLTT	oxygen- and capacity limited thermal tolerance
O. mykiss	*Oncorhynchus mykiss*, rainbow trout
P_{air}	partial pressure of oxygen
PCO_2	partial pressure of carbon dioxide
PO_2	partial pressure of oxygen
P_{water}	water vapor pressure
RCP	Representative Concentration Pathway
RF	Rijpfjorden
R. canadum	*Rachycentron canadum*, cobia
SDA	specific dynamic action
SGR	specific growth rate
SMR	standard metabolic rate
SF	stomach filling
S. caurinus	*Sebastes caurinus*, copper rockfish
S. mystinus	*Sebastes mystinus*, blue rockfish
T_{opt}	optimum temperature for growth
T_{pej}	pejus ("getting worse") temperature
T_{crit}	critical temperature
TSB	time between U_{gait} & U_{crit} ("time spent bursting")
$TSB_{anaerob}$	estimated proportion of anaerobic metabolism
U_{crit}	critical swimming speed (maximum achievable swimming speed)
U_{gait}	gait transition speed (here: maximum sustainable swimming speed)
UiT	University of Tromsø
UNFCCC	United Nations Framework Convention on Climate Change
WSC	West Spitsbergen Current
Z. viviparus	*Zoarces viviparus*, common eelpout
αO_2	oxygen solubility in water

Summary

The anthropogenic use of fossil fuels since the beginning of the industrial revolution and the associated, unprecedented emission of CO_2 into the atmosphere entails progressively rising temperatures. Due to interactions between the atmosphere and water surface, the oceans are experiencing a distinct warming trend along with rising PCO_2 levels with pronounced regional differences. The Arctic Ocean is projected to warm at the highest rate. Low water temperatures in the Arctic and the progressive freshening of surface waters in response to a decreasing sea ice cover further enhance the local solubility of CO_2.
Species inhabiting the Arctic are well-adapted to a narrow range of low temperatures and, therefore, are expected to possess limited acclimation capacities to rapidly changing abiotic conditions. Furthermore, rising water temperatures are progressively provoking invasions of boreal ectotherms into Arctic waters, likely causing tremendous shifts in the Arctic ecosystem due to different species-specific thermal sensitivities. Thus, the present PhD project focused on two gadoid fish species co-occurring in Svalbard waters: the Arctic endemic Polar cod (*Boreogadus saida*), an important species in the Arctic foodweb due to its high energy content and large standing stock biomass, and the boreal Atlantic cod (*Gadus morhua*), which is invading Arctic waters and recently established its year-round occurrence in the vicinity of Svalbard.

In this thesis, I investigated a set of whole-animal routine performance parameters for both *B. saida* and *G. morhua* after long-term acclimation to control and projected temperature (*B. saida*: 0, 3, 6, 8°C; *G. morhua*: 3, 8, 12, 16°C) and PCO_2 (390 and 1170 µatm) combinations in order to estimate their future relative competitive strength. The investigated routine performance parameters comprised somatic growth (specific growth rate, SGR), food consumption (F) and food conversion efficiency (FCE), body index parameters (condition factor, CF; hepatosomatic index, HSI; gonadosomatic index, GSI) and standard metabolic rate (SMR).

Routine performance parameters determined after long-term exposure to a set of control and projected elevated temperatures emphasized the adaptation of *B. saida* to cold temperatures: *B. saida* showed a low SMR at 0°C, which likely resulted in the low F determined at that temperature that entailed low costs for digestion (SDA magnitude). A significantly elevated FCE of *B. saida* at 0°C that progressively decreased throughout the

range of investigated acclimation temperatures indicated that digestive processes were well-adapted to low habitat temperatures. Furthermore, GSI values were highest at 0°C. Despite of *B. saida* being well-adapted to temperatures around the freezing-point, a peak in SGR when fed *ad libitum* and a maximized aerobic scope for exercise (AS_{ex}) suggested an optimum temperature for this species of 6°C. Long-term acclimation at 8°C, in turn, revealed limitations in the acclimation capacity of *B. saida* expressed by a significantly enhanced SMR likely evoked by an elevated mitochondrial proton leak that further entailed an impairment in SGR. Accordingly, 8°C was classified as the long-term upper pejus temperature (T_{pej}) for this species.

In contrast, *G. morhua* showed improving performance at higher acclimation temperatures. A lower T_{pej} was identified at 3°C, based on a reduced FCE compared to 8 – 16°C. Any other performance parameter recorded for *G. morhua* increased progressively with acclimation temperature within the experimental range, emphasizing the eurythermal physiology of *G. morhua* compared to the rather stenothermal *B. saida*.

Compared to the distinct impact of temperature, effects of elevated PCO_2 levels on routine performance parameters were rather small in both species. High CO_2 conditions at 0 – 6°C caused a non-significant impairment in the SGR of *B. saida* that was not observed in *G. morhua*. This may be indicative of a lower sensitivity of *G. morhua* to near-future elevated PCO_2 levels compared to *B. saida*. *G. morhua* showed a significantly elevated HSI under high PCO_2 levels at the highest acclimation temperature (16°C), which may indicate a shift in metabolic fuels in response to warm-acclimation and thereby elevated thermal sensitivity under hypercapnic conditions.

Observations of routine performance parameters suggest a declining competitive strength of *B. saida* in Svalbard waters unter future climate change scenarios, especially considering a parallel, progressively improving performance of the boreal *G. morhua*.

In addition to routine performance, I determined maximum performance parameters of *B. saida* at projected future abiotic (rising temperatures and PCO_2 levels) and biotic (food deprivation) scenarios in order to comprehensively unravel limitations in the acclimation capacity of the Arctic keystone species under future ocean conditions. Measurements of maximum performance of *B. saida* involved maximum sustainable swimming speed (U_{gait}) (exclusively fueled by aerobic metabolism) and critical swimming speed (U_{crit}) (maximum achievable swimming speed partially fueled by anaerobic metabolism) as well as the

maximum metabolic rate associated with U_{crit} (MMR_{ex}). Furthermore, the anaerobic swimming capacity of *B. saida* was estimated based on burst-and-coast swimming events.

The MMR_{ex} of *B. saida* increased with acclimation temperature and – combined with fully compensated SMR values at all acclimation temperatures except 8°C – resulted in a peak AS_{ex} at 6°C. The maximized aerobic performance, however, was neither reflected in the U_{gait} nor in the U_{crit} of *B. saida*. Instead, *B. saida* maintained a stable swimming performance throughout the thermal range investigated in this study. Nevertheless, a decreasing maximum swimming efficiency (E_{max}, defined as U_{crit} MMR_{ex}^{-1}) indicated progressive performance limitations at water temperatures ≥ 6°C under normocapnia and ≥ 3°C under hypercapnia.

Long-term exposure to near-future PCO_2 levels resulted in a distinct impact on the maximum performance of *B. saida*: The maximum swimming performance (both U_{gait} and U_{crit}) of *B. saida* was depressed despite a significantly elevated MMR_{ex} and thereby AS_{ex}. Accordingly, E_{max} was significantly impaired under hypercapnic conditions. Aerobic processes other than exercise, therefore, are hypothesized to be elevated during maximum performance under hypercapnia resulting in downstream effects on swimming performance.

Rapidly changing abiotic conditions in the Arctic are projected to decrease the abundance of prey organisms for *B. saida*. Accordingly, I investigated maximum performance of fed and food-deprived *B. saida* at control (0°C) and elevated (6°C) temperatures. At 0°C, the MMR_{ex} of unfed fish was comparable to the maximum metabolic rate of fed fish (MMR_{ex+dig}). Under this cold temperature, simultaneous energetic demands of exercise and digestion caused a reduction in U_{gait} and U_{crit} presumably caused by an elevated blood flow to the digestive system resulting in an oxygen limitation of the swimming muscle of fed fish. At the elevated temperature of 6°C, however, MMR_{ex+dig} was significantly higher than MMR_{ex}. Instead of an additive effect of aerobic demands for exercise and digestion, the swimming capacity of fed fish was significantly elevated. This result suggests that - unlike the situation at the cold acclimation temperature - food-deprived fish were not able to fully exploit their muscular capacities at 6°C, potentially evoked by nutrient limitation of the swimming muscle.

Burst-and-coast swimming, considered a measure for the anaerobic swimming capacity in this study, generally plays an essential role during predator escape reactions. The burst swimming performance of *B. saida* was lower compared to more active pelagic species, reflecting this species' strategy to avoid predator encounters by hiding in hardly accessible habitats characterized by low temperatures. Similar to aerobic swimming performance, burst swimming was not affected by elevated temperatures, while both near-future elevated PCO_2 levels and food deprivation caused a reduction in the burst swimming performance of *B. saida*.
Accordingly, results obtained for both aerobic and anaerobic swimming capacities indicate a distinct fitness impairment of *B. saida* under projected water conditions.

In conclusion, the results of the present thesis revealed limitations in the acclimation capacity of *B. saida* at 8°C. Concomitantly rising PCO_2 levels and decreasing prey densities for *B. saida* resulted in a reduced aerobic and anaerobic swimming performance. Consequences of the impaired swimming performance are expected to be amplified during the progressive retreat of sea ice cover and the associated decrease in shelter, low-temperature refuges and under-ice prey abundance. Together with expected prolonged forage excursions triggered by reduced prey densities and rising metabolic costs, these future developments pose enhanced risks for predation of *B. saida* and its future stock size, especially when considering the increasing performance of the boreal *G. morhua* in progressively warming Svalbard waters. Therefore, results of this thesis indicate high risk for a continually decreasing abundance of the endemic *B. saida* in the vicinity of Svalbard with potentially tremendous implications for the Arctic food web and ecosystem.

Zusammenfassung

Die anthropogene Nutzung fossiler Brennstoffe seit Beginn der industriellen Revolution und die damit einhergehende beispiellose Emission von CO_2 in die Atmosphäre führen zu steigenden Temperaturen. Aufgrund von Wechselwirkungen zwischen Atmosphäre und Wasseroberfläche erfahren die Weltmeere ebenfalls einen ausgeprägten Temperaturanstieg sowie steigende PCO_2-Werte mit deutlichen regionalen Unterschieden. Für den Arktischen Ozean wird die höchste Erwärmungsrate vorhergesagt. Darüber hinaus begünstigen die niedrigen Wassertemperaturen in der Arktis und die durch schmelzendes Meereis fortschreitend abnehmende Salinität des Oberflächenwassers die Löslichkeit von CO_2 im Arktischen Ozean.

Endemische Arten in der Arktis sind gut an einen schmalen Bereich niedriger Temperaturen angepasst und dürften infolgedessen nur über begrenzte Kapazitäten verfügen, um sich an schnell ändernde abiotische Bedingungen anzupassen. Darüber hinaus verursachen steigende Wassertemperaturen zunehmend ein Eindringen borealer ektothermer Arten in arktische Gewässer, was aufgrund unterschiedlicher artspezifischer Temperatursensitivitäten voraussichtlich erhebliche Verschiebungen im arktischen Ökosystem nach sich zieht. Demnach konzentrierte sich die vorliegende Studie auf zwei Arten von Gadidae, welche beide in den Gewässern um Spitzbergen auftreten: den arktisch-endemischen Polardorsch (*Boreogadus saida*), der aufgrund seines hohen Energiegehalts und einer hohen Abundanz eine wichtige Stellung im arktischen Nahrungsnetz einnimmt, sowie den borealen Atlantischen Kabeljau (*Gadus morhua*), der in arktische Gewässer vordringt und seit kurzer Zeit ganzjährig die Gewässer um Spitzbergen besiedelt.

In der vorliegenden Arbeit untersuchte ich mehrere Ganztier-Routineparameter von *B. saida* und *G. morhua* nach Langzeitakklimierung an Kontroll- und vorhergesagte Bedingungen für Temperatur (*B. saida*: 0, 3, 6, 8°C; *G. morhua*: 3, 8, 12, 16°C) und PCO_2 (390 und 1170 µatm), um ihre zukünftige relative Konkurrenzstärke einzuschätzen. Die gemessenen Routineparameter umfassten somatisches Wachstum (spezifische Wachstumsrate, SGR), Futteraufnahme (F) und Futterverwertungseffizienz (FCE), Körperindexparameter (Konditionsfaktor, CF; hepatosomatischer Index, HSI; gonadosomatischer Index, GSI), sowie die Standard-Stoffwechselrate (SMR).

Die Routineparameter, die nach Langzeitexponierung an Kontroll- und vorhergesagte, erhöhte Temperaturen gemessen wurden, bestätigten die Kälteanpassung von *B. saida*: *B. saida* zeigte bei 0°C eine niedrige SMR, was wahrscheinlich zu der niedrigen Futteraufnahme bei dieser Temperatur führte, wodurch nur geringe Kosten für die Verdauung erforderlich wurden. Die signifikant erhöhte FCE von *B. saida* bei 0°C, die mit steigender Akklimierungstemperatur fortwährend abnahm, deutete darauf hin, dass Verdauungsprozesse gut an die niedrigen Temperaturen im Lebensraum von *B. saida* angepasst sind. Darüber hinaus waren die GSI-Werte bei 0°C am höchsten. Trotz der guten Anpassung an Temperaturen um den Gefrierpunkt deuteten ein Höchstwert in der SGR in *ad libitum* gefütterten Tieren, sowie maximierte aerobe Kapazitäten für Aktivität (AS_{ex}) auf Optimalbedingungen für *B. saida* bei 6°C hin. Hingegen zeigten sich nach Langzeitexponierung auf 8°C Einschränkungen in der Akklimierungskapazität von *B. saida*, die in einer signifikant erhöhten SMR sichtbar wurden. Die erhöhte SMR konnte mit einem verstärkten Protonenleck in der Mitochondrienmembran in Verbindung gebracht werden, welches weiterhin einen Abfall in der SGR verursachte. Dementsprechend wurde 8°C als langfristige obere Pejus-Temperatur (T_{pej}) dieser Art eingestuft.

Im Gegensatz zu den Ergebnissen bei *B. saida* zeigte *G. morhua* eine verbesserte Leistung mit höherer Akklimierungstemperatur. Die untere T_{pej} von *G. morhua* wurde bei 3°C identifiziert, basierend auf einer verringerten FCE verglichen mit einer stabilen FCE im Temperaturbereich zwischen 8 – 16°C. Alle weiteren Routineparameter, die für *G. morhua* ermittelt wurden, stiegen fortwährend mit der Akklimierungstemperatur an, wodurch die eurytherme Physiologie von *G. morhua* im Vergleich zu dem eher stenothermen *B. saida* betont wurde.

Verglichen mit dem deutlichen Einfluss von Temperatur waren die Auswirkungen erhöhter PCO_2-Werte auf die Routineparameter beider Arten eher gering. Hoch-CO_2-Bedingungen führten zu einer nicht signifikanten Beeinträchtigung der SGR von *B. saida* bei 0 – 6°C, die bei *G. morhua* nicht beobachtet wurde. Dies könnte auf eine - im Vergleich zu *B. saida* - geringere Sensitivität von *G. morhua* für zukünftig erhöhten PCO_2-Werte hindeuten. Unter Hoch-PCO_2-Werten zeigte *G. morhua* bei der höchsten Akklimierungstemperatur (16°C) einen signifikant erhöhten HSI. Dies könnte auf eine Verschiebung der Stoffwechselwege als Reaktion auf die Warmakklimierung und somit auf eine erhöhte Temperatursensitivität unter hyperkapnischen Bedingungen hinweisen.

Beobachtungen von Routineparametern deuten auf einen Rückgang in der Konkurrenzstärke von *B. saida* unter zukünftigen Wasserbedingungen hin, insbesondere

im Hinblick auf eine zunehmende Leistungssteigerung des borealen *G. morhua* in den Gewässern um Spitzbergen.

Zusätzlich zu Routineparametern konzentrierte ich mich auf maximale Leistungsparameter von *B. saida* unter vorhergesagten abiotischen (steigende Temperaturen und PCO_2-Werte), sowie biotischen (Nahrungsknappheit) Szenarien, um Begrenzungen in der Akklimierungskapazität dieser arktischen Schlüsselart an zukünftige Meeresbedingungen umfassend aufzudecken. Messungen der Maximalleistung von *B. saida* umfassten die maximale nachhaltige Schwimmgeschwindigkeit (U_{gait}) (ausschließlich durch aeroben Stoffwechsel betrieben) und die kritische Schwimmgeschwindigkeit (U_{crit}) (maximal erreichbare Schwimmgeschwindigkeit, die teilweise durch den anaeroben Stoffwechsel betrieben wird) sowie die mit U_{crit} verbundene maximale Stoffwechselrate (MMR_{ex}). Darüber hinaus wurde die anaerobe Schwimmkapazität von *B. saida* anhand von gepulstem Schwimmverhalten eingeschätzt.

Die maximale Stoffwechselrate von *B. saida* stieg mit der Akklimationstemperatur, was - in Kombination mit der vollständig kompensierten SMR bei allen Temperaturen außer 8°C - zu einer maximierten AS_{ex} bei 6°C führte. Die maximierte AS_{ex} schlug sich jedoch weder in der U_{gait} noch in der U_{crit} von *B. saida* nieder. Stattdessen wurde eine stabile Schwimmleistung über den gesamten untersuchten Temperaturbereich festgestellt. Eine abnehmende Effizienz der maximalen Schwimmleistung (E_{max}, definiert als $U_{crit}\ MMR_{ex}^{-1}$) deutete jedoch auf steigende Leistungslimitierungen bei Wassertemperaturen ≥ 6°C unter Normokapnie bzw. ≥ 3°C unter Hyperkapnie hin.
Langzeitexponierung an zukünftige PCO_2-Werte hatte einen erheblichen Einfluss auf die maximale Schwimmleistung von *B. saida*: Die maximale Schwimmleistung (sowohl U_{gait} als auch U_{crit}) war unter zukünftigen CO_2-Bedingungen trotz einer deutlich erhöhten MMR_{ex} – und damit AS – beeinträchtigt. Dementsprechend war auch die E_{max} unter hyperkapnischen Bedingungen signifikant verringert. Es wird daher angenommen, dass die nachteiligen Auswirkungen auf die Schwimmleistung durch andere hochregulierte aerobe Prozesse während maximaler aerober Anstrengung unter Hyperkapnie hervorgerufen wurden.

Sich rasch verändernde abiotische Bedingungen in der Arktis ziehen eine Reduktion in der Abundanz der Beuteorganismen von *B. saida* nach sich. Aus diesem Grund untersuchte ich

die Maximalleistung von ungefütterten und gefütterten *B. saida* sowohl bei Kontrolltemperaturen (0°C), als auch bei erhöhten Temperaturen (6°C). Bei 0°C war die MMR_{ex} ungefütterter Fische vergleichbar mit der maximalen Stoffwechselrate gefütterter Fische (MMR_{ex+dig}). Folglich führten gleichzeitige energetische Bedürfnisse von Bewegung und Verdauung bei dieser Temperatur zu einer Verminderung von U_{gait} und U_{crit}. Dies war höchstwahrscheinlich auf einen erhöhten Blutfluss im Verdauungssystem gefütterter Fische zurückzuführen, der in einer Sauerstofflimitierung des Schwimmmuskels dieser Tiere resultierte. Bei der erhöhten Temperatur war MMR_{ex+dig} jedoch signifikant höher als MMR_{ex}. Anstelle eines additiven Effekts des zeitgleichen aeroben Energiebedarfs von Bewegung und Verdauung war die Schwimmkapazität gefütterter Fische signifikant erhöht. Dieses Ergebnis deutet darauf hin, dass ungefütterte Fische bei 6°C - im Gegensatz zur Situation bei 0°C - nicht ihre maximale Muskelkapazität ausschöpfen konnten, was augenscheinlich mit einer Nährstofflimitierung des Schwimmmuskels zu erklären ist.

Gepulste Schwimmbewegungen, die in dieser Studie als Maß für die anaerobe Schwimmkapazität von *B. saida* angesehen wurden, spielen gewöhnlich eine entscheidende Rolle bei der Flucht vor Fraßfeinden. Die Kapazität für gepulste Schwimmbewegungen erwies sich bei *B. saida* im Vergleich zu aktiveren, pelagisch lebenden Arten als gering. Dies spiegelt die besondere Strategie dieser Art wider, die Begegnungen mit Fraßfeinden zu vermeiden, indem sie sich in schwer zugängliche und/oder durch niedrige Temperaturen gekennzeichnete Lebensräume zurückziehen. Ähnlich dem aeroben Schwimmen wurde anaerobes Schwimmen nicht durch zukünftig erhöhte Temperaturen beeinflusst, während sowohl erhöhte PCO_2-Werte als auch Futterentzug die Kapazität für gepulste Schwimmbewegungen von *B. saida* verringerten. Demnach lässt sich anhand der Ergebnisse sowohl zum aeroben als auch zum anaeroben Schwimmen eine deutliche Beeinträchtigung der Fitness von *B. saida* unter zukünftigen Wasserbedingungen ableiten.

Zusammenfassend zeigten die Ergebnisse der vorliegenden Arbeit Limitierungen in der Akklimierungskapazität von *B. saida* bei 8°C. Gleichzeitig steigende PCO_2-Werte und eine rückläufige Abundanz der Beuteorganismen von *B. saida* führten zu einer Verringerung der aeroben und anaeroben maximalen Schwimmleistung dieser Art. Es ist zu erwarten, dass die Auswirkungen einer beeinträchtigten Schwimmleistung bei einem fortschreitenden Rückgang des Meereises verstärkt werden, da dies mit schwindenden

Unterschlupfmöglichkeiten mit niedrigen Temperaturen sowie sinkenden Verfügbarkeiten an Untereis-Beuteorganismen verbunden ist. Zusammen mit zu erwartenden längeren Jagdausflügen, die durch verringerte Beutedichten und steigende Stoffwechselkosten ausgelöst werden könnten, stellen diese zukünftigen Entwicklungen ein erhöhtes Risiko für *B. saida* dar, selbst Prädatoren zum Opfer zu fallen. Dieses Risiko wird insbesondere deutlich, ruft man sich die zunehmende Leistung des borealen *G. morhua* bei einer fortwährend schrittweisen Erwärmung der Gewässer um Spitzbergen vor Augen. Somit deuten die Ergebnisse der vorliegenden Arbeit auf eine hohe Wahrscheinlichkeit für eine kontinuierliche Abnahme der Abundanz von *B. saida* in den Gewässern um Spitzbergen hin, mit gegebenenfalls erheblichen Auswirkungen auf das Nahrungsnetz und somit das Ökosystem der Arktis.

1 Introduction

Oceans are currently experiencing a rise in PCO_2 levels which occurs in parallel to a distinct increase in temperature as a result of the anthropogenic use of fossil fuels. Compared to global average, changes in abiotic conditions are projected to occur in a higher magnitude and rate in the Arctic Ocean that is inhabited by species adapted to a naturally stable environment. Accordingly, changes in abiotic conditions are expected to entail tremendous ecological consequences that most likely will also affect societal and economic levels.

The present thesis focusses on the performance and acclimation capacities of two co-occurring Arctic fish species, the Arctic key species Polar cod (*Boreogadus saida*) and the invading boreal Atlantic cod (*Gadus morhua*) long-term exposed to predicted ocean acidification and warming (OAW).

1.1 Global climate change and its impact on the oceans

Human activities have been identified with 95 % certainty as the main reason for the currently ongoing unprecedentedly rapid climate change (IPCC 2014), motivating scientists to establish a new geological epoch, the Anthropogene (Crutzen 2002), a period formally starting from the mid-twentieth century (Waters et al. 2016).

1.1.1 Climate change from the beginning of the industrial revolution to the present

Since the beginning of the industrial revolution, the use of fossil fuels rose extensively with recent emissions of greenhouse gases (GHG) such as CO_2, CH_4, N_2O as well as chlorofluorocarbons being the highest in history (IPCC 2014). In particular, global average atmospheric PCO_2 levels increased from 278 ppm in 1750 to 390.5 ppm in 2011 (FIG. 1), thereby exceeding values experienced on the Earth for approximately the past 800,000 years (Rhein et al. 2013). Together with other GHGs and aerosols, CO_2 contributes to imbalances in the Earth`s radiative energy budget by aggravating the reflection of solar energy back to space, causing heat accumulation and thereby a warming effect in the atmosphere (Cubasch et al. 2013) as well as in the ocean (Rhein et al. 2013). Despite substantial decadal and interannual variability, an increase in average global land and ocean surface temperature of 0.78°C (0.72 – 0.85°C) was detected between the periods of 1850 – 1900 and 2003 – 2012 (Hartmann et al. 2013) (FIG. 2).

1.1.2 Projected climate change for the year 2100

The Intergovernmental Panel on Climate Change (IPCC) generated four Representative Concentration Pathways (RCPs) that consider different potential future scenarios concerning the magnitude and the range of GHG emissions. Based on the respective RCPs, consequences on the climate system were estimated and likely entailing environmental impacts and adaptation capacities were described. In particular, the RCPs cover a mitigation scenario demanding substantial net negative emissions (RCP2.6), intermediate scenarios with or without additional efforts to constrain emissions (RCP4.5 and RCP6.0, respectively) and one scenario with high GHG emissions (RCP8.5). For the year 2100, the different RCPs predict atmospheric PCO_2 levels of 420 – 940 ppm (RCP2.6 and RCP8.5) (IPCC 2013, Annex II) (FIG. 1).

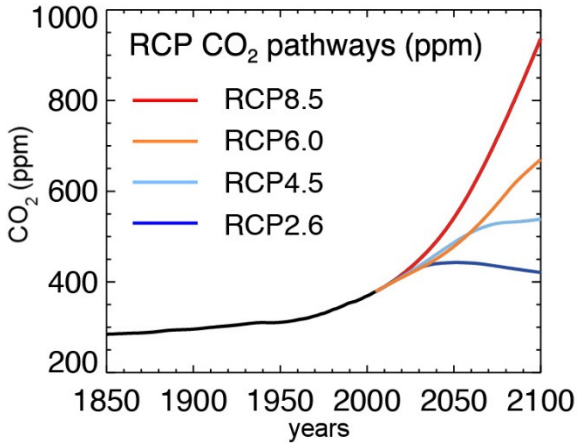

FIGURE 1. Observed historical (black line) and projected future changes (line color according to RCP pathways) in atmospheric PCO_2 levels (Ciais et al. 2013).

A temperature increase of 0.3 – 0.7°C is projected for the year 2050 relative to the period 1986 – 2005 irrespective of the different emission scenarios. From the mid-21st century on, however, emission scenarios differ increasingly in their projections concerning a further rise in global surface temperature from different sensitivities in climate models in response to the respective forcing (IPCC 2014). Thus, an increase in global mean surface temperature of 1.0°C (RCP2.6) to 3.7°C (RCP8.5) can be expected for the end of this century (2081 – 2100) in relation to the reference period 1986 – 2005 (Collins et al. 2013) (FIG. 2).

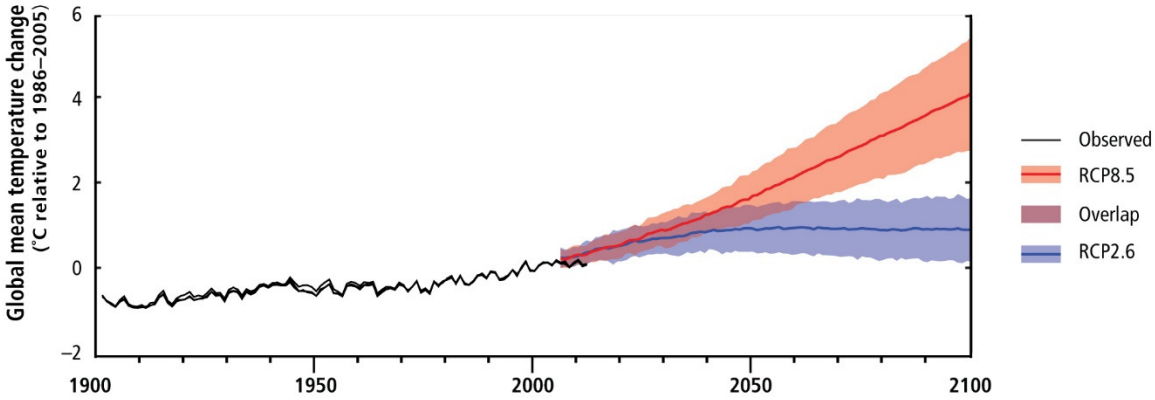

FIGURE 2. Observed historical (black line) and projected future changes in annual average surface temperature (blue and red lines and shadings according to RCP2.6 and RCP8.5, respectively; means ± standard deviation) relative to 1986-2005 (x-axis = time (years)) (IPCC 2014).

In order to substantially attenuate impacts of climate change on the earth system, parties of the United Nations Framework Convention on Climate Change (UNFCCC) agreed on pursuing efforts to limit global warming to a mean surface temperature increase of 1.5°C above pre-industrial levels in the longer term ("Paris Agreement") (Allen et al. 2018). In 2017, human-induced global warming reached 1.0°C (0.8 – 1.2°C) above pre-industrial levels (Allen et al. 2018). If warming continues at the current rate, 1.5°C above pre-industrial conditions are projected to be reached by 2030 – 2052 (IPCC 2018). Accordingly, unprecedented and immediate global collective efforts are required to mitigate GHGs emissions in order to meet the "1.5°C goal" (Hoegh-Guldberg et al. 2018).

1.1.3 Climate change in the oceans

Consistent with atmospheric CO_2 observations, PCO_2 levels are currently increasing in the oceans (Le Quéré et al. 2010). According to reconstructions of the atmospheric CO_2 history, less than half of the CO_2 released by human activities remains in the atmosphere (Sabine et al. 2004). Roughly one third of total emissions since the beginning of the industrial revolution have been dissolved in the oceans (Khatiwala et al. 2013). Thus, the oceans absorbed ~ 155 PgC of anthropogenic CO_2 produced between 1750 and 2010 (Khatiwala et al. 2013) with distinct regional variations: Despite its comparatively small dimensions, the North Atlantic stores 23 % of the total oceanic anthropogenic CO_2 inventory, while the whole Southern Hemisphere oceans with a distinctly larger area only absorbs approx. 60 % (Sabine et al. 2004).

In sea water, dissolved carbon dioxide ($CO_{2\,(aq)}$) forms carbonic acid (H_2CO_3), which rapidly dissociates into bicarbonate (HCO_3^-) and carbonate ions (CO_3^{2-}) according to the following equation (Feely et al. 2009):

$$CO_{2\,(aq)} + H_2O \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^- \rightleftarrows 2H^+ + CO_3^{2-}$$

Under typical sea water conditions, ~ 90 % of the total dissolved inorganic carbon (DIC) is represented by HCO_3^-, ~ 9 % by CO_3^{2-} and only ~ 1 % exists as $CO_{2\,(aq)}$ and H_2CO_3. Elevated atmospheric PCO_2 due to human activities cause higher rates of CO_2 uptake by the oceans resulting in fundamental changes in sea water chemistry. The additional $CO_{2\,(aq)}$ entails a higher formation of HCO_3^- and hydrogen ions (H^+) (Feely et al. 2009). Thus, the increasing uptake of anthropogenic CO_2 by the oceans over an extended period causes an elevation in H^+ concentration, corresponding to a decrease in sea water pH – a phenomenon called ocean acidification (OA) (Feely et al. 2009, Rhein et al. 2013). Within the past two and a half centuries, the average ocean surface pH was reduced from 8.2 to 8.1 (Caldeira and Wickett 2003, Orr et al. 2005). According to projections of all four RCPs, the oceans are expected to absorb even higher amounts of anthropogenic produced CO_2 in the future (Rhein et al. 2013) entailing a further decrease in surface water pH of up to 0.4 units by the year 2100 (Caldeira and Wickett 2003, Feely et al. 2009), which represents pH values lower than experienced in the past 50 million years (Rhein et al. 2013). Further, by reacting with CO_3^{2-}, elevated H^+ concentrations in response to increasing levels of $CO_{2\,(aq)}$ induce a decrease in the calcium carbonate ($CaCO_3$) minerals calcite and aragonite, affecting important biological processes (e.g. shell formation of corals, plankton and shellfish), thereby triggering a cascade of detrimental effects on whole ecosystems (Feely et al. 2009).

The role of the oceans as a carbon sink contributes to alleviate global warming. However, the constant exchange between atmosphere and sea surface entails also a warming trend in the oceans that underlies distinct local and temporal fluctuations due to variations in ocean currents (Rhein et al. 2013). Despite substantial year-to-year and decadal fluctuations in temperature, the global mean upper ocean temperature (up to 75 m depth) experienced a clear warming trend of 0.11°C per decade between 1971 and 2010. This trend is less pronounced with increasing water depth (0.04°C/decade by 200 m, < 0.02°C/decade by 500 m) (Rhein et al. 2013). By the end of this century, RCP2.6 and RCP8.5 predict the ocean

surface temperature to increase by 1°C and > 3°C, respectively, relative to 1986 – 2005. Even at a depth of 1000 m, sea water temperatures are predicted to increase between 0.5°C (RCP2.6) and 1.5°C (RCP8.5) within the same time frame (Collins et al. 2013). While the upper levels of the ocean adjust comparably quickly (on the scale of decades) to external climate forcing, the response of the deep sea happens on larger time scales (centuries to millennia) due to slow circulations (Held et al. 2010). Accordingly, in the theoretical case of immediate cease of GHG emissions, surface sea temperatures would continue to rise for another decade, while deep sea water masses would continue to warm for centuries to millennia (Rhein et al. 2013).

Despite a reduced solubility of CO_2 in warmer waters (Maier-Reimer et al. 1996), the oceans still experience an ongoing decrease in pH due to an elevated formation of CO_3^{2-} from HCO_3^- at higher temperatures and a corresponding elevated release of H^+ (Rhein et al. 2013).

1.2 The Arctic under climate change

The Arctic region comprises the area north of 66°N. This area is characterized by historically low natural fluctuations in abiotic conditions (Clarke 1983).

1.2.1 Ocean acidification and warming (OAW) in the Arctic

The Arctic is projected to experience the globally highest rate of warming caused by anthropogenic release of GHGs (Larsen et al. 2014) (FIG. 3).

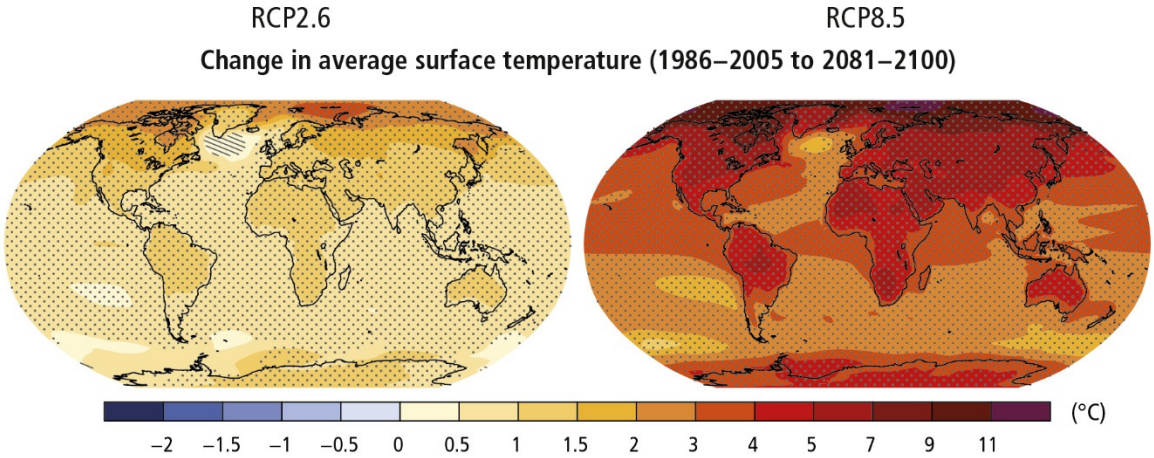

FIGURE 3. Global overview of projected changes in annual average surface temperature (left: RCP2.6, right: RCP8.5) for the period 2081 – 2100 relative to 1986 – 2005 (IPCC 2014).

This phenomenon termed Polar amplification arises from the retreat in sea ice in response to elevated temperatures of northwards directed Atlantic water masses and a concomitant decrease in albedo entailing a further local increase in atmospheric and water temperature, which in turn accelerates the decrease in sea ice (Masson-Delmotte et al. 2013). Accordingly, atmospheric winter temperature over the Arctic Ocean is projected to increase by ~ 4 – 16°C by the year 2100 relative to 1986 – 2005 depending on the emission scenario. In contrast, a summer temperature rise of only ~ 1 – 5°C is expected over the Arctic sea within the same time frame (IPCC 2013, Annex I) (FIG. 4). The amplification of the Arctic winter warming is mostly attributed to enhanced wintertime sea ice - infrared radiation feedbacks (Bintanja and van der Linden 2013).

As a consequence of Polar amplification, the Arctic Ocean is projected to be nearly ice-free in September before the year 2050 according to the business-as-usual GHG emission scenario (RCP8.5) (Collins et al. 2013).

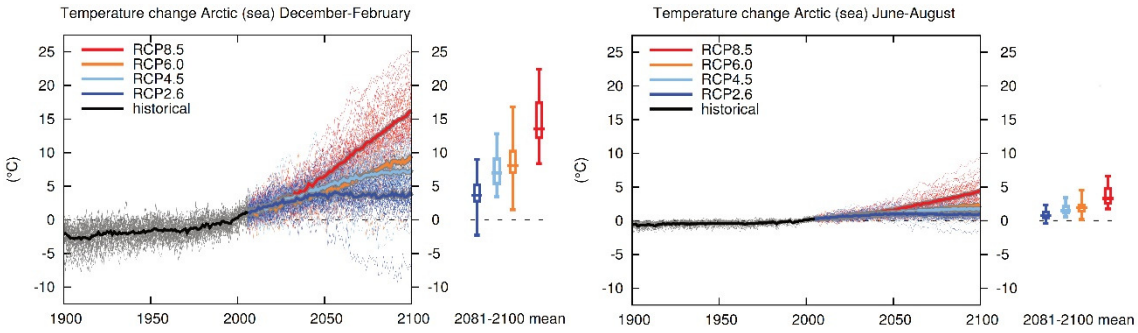

FIGURE 4. Time series of atmospheric temperature change relative to 1986 – 2005 over the Arctic sea in winter (left) and summer (right). Black line: historical observations/reconstructions; colored lines: projected changes separated by RCP pathways. Thick lines represent multi-model means, thin lines represent one ensemble member per model within the RCPs. Right-hand side: medians and 95 percentiles for projected temperature changes for the period 2081 – 2100 by the RCPs (IPCC 2013, Annex I).

The solubility of CO_2 is high in cold Arctic water masses (Fransson et al. 2009). Further, the ongoing freshening of Arctic surface waters and the facilitated gas exchange between atmosphere and sea water due to sea ice retreat counteract a general decreasing solubility of CO_2 in warming waters (Steinacher et al. 2009). Therefore, the Arctic Ocean is expected to experience an increase in pCO_2 levels from 400 to 1,370 µatm (IPCC 2014), corresponding to the worldwide largest decrease in pH (-0.45 units within the 21th century) (Steinacher et al. 2009) (FIG. 5).

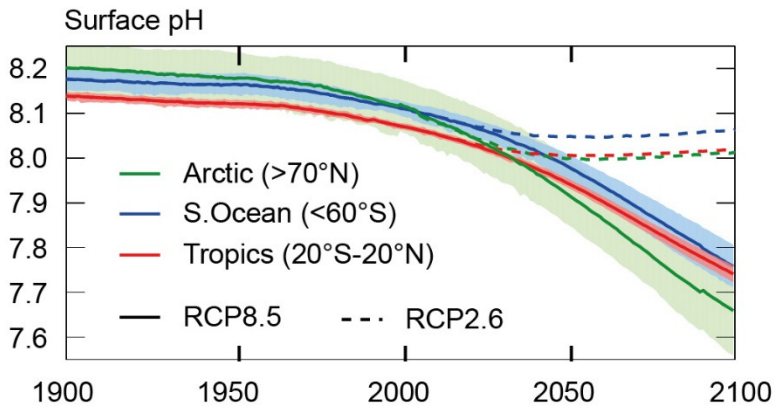

FIGURE 5. Projected decrease in surface pH until the year 2100 separated by three major climate zones. Solid lines and shadings represent mean values and the range of models within RCP8.5. Dashed lines represent mean surface pH projections according to RCP2.6 (x-axis = time (years)) (Ciais et al. 2013).

1.2.2 Area of focus within the Arctic

The focus area of the present thesis are the waters in the vicinity of the Svalbard archipelago in the Arctic Ocean (FIG. 8). Svalbard is located approximately halfway between continental Norway and the North Pole at 74 – 81°N and 10 – 35°E (Klemsdal 2010). Along the west coast of Svalbard, warm Atlantic water masses are entering the Arctic through the Fram Strait in the West Spitsbergen Current (WSC). The water temperature of the WSC has increased by about 0.3°C per decade since 1979, resulting in a northeastward retreat of the ice edge north of Svalbard (Onarheim et al. 2014). The east coast of Svalbard is influenced by cold Arctic water masses from northeast. This southward current passes the southern tip of Svalbard and continues as a northward coastal current along Western Svalbard. Historically, this cold current caused the fjords on the west coast to be frozen in winter, a pattern not observed in the past decade anymore (Misund et al. 2016). More precisely, maximum water temperatures within Isfjorden and Grønfjorden located at the west coast of Svalbard increased by ~ 2°C during the past century (Pavlov et al. 2013), yet with distinct annual fluctuations (mean temperature at the mouth of Isfjorden: 1.5 and 5.0°C in 1989 and 2013, respectively) (Misund et al. 2016).

The waters around Svalbard are rich fishing grounds (Hønneland 1998) and accommodate the world's northernmost regular fishery zone, termed Svalbard zone, that covers an area of 750,000 km^2 (Misund et al. 2016). Fishing activity within the Svalbard zone is most intense around Bear Island and in the area between Svalbard and Bear Island, as well as

along the continental shelf and within the large fjord systems on the west coast of Svalbard depending on the target species (Misund et al. 2016).

1.3 Marine fish species – indicators for climate change in the Arctic

Ectotherms such as fish tolerate a species-specific range of temperatures that support the functionality of their molecular, cellular and systemic processes (Pörtner and Farrell 2008). Accordingly, temperature governs the spatial distribution of ectotherm fish species (Magnuson et al. 1979). In order to preserve organismic performance, fish respond to rising temperatures with distribution shifts along latitudes (e.g. Perry et al. 2005) or to greater depths (Dulvy et al. 2008), resulting in the retreat of resident species that may even lead to local extinctions at their southern distribution limits (Pörtner et al. 2017). Due to the high rate of climate change, the Arctic is expected to experience the globally largest species turnover (Cheung et al. 2009). In fact, between 2004 and 2012, boreal fish communities shifted their distribution ranges into the Northern Barents Sea that was previously dominated by Arctic fish communities with a pace roughly four times the global average (Fossheim et al. 2015). Likewise, poleward directed shifts in distribution evoked by ocean warming are observed for fish species in other Arctic regions, such as the Bering Sea (Grebmeier et al. 2006) and off the west coast of Greenland (Fossheim et al. 2015). Nevertheless, sensitivities to increasing water temperatures are species-specific and therefore entail relative changes in performance (Pörtner and Farrell 2008, Pörtner 2012) resulting in differential risk for local extinctions and accordingly in changes in community composition, species interaction and food web dynamics (Pörtner et al. 2014).

Ultimately, both successful settlement of invading boreal fish species in Arctic waters as well as the persistence of endemic Arctic species in their southern distribution areas is not solely determined by abiotic, but also by biotic factors such as the quantity and quality of food, the local predation risk and competition pressure as well as suitable spawning areas that ensure a consistent supply of juveniles (Drinkwater 2005, Fossheim et al. 2015). Although feeding areas are expected to enlarge in the Arctic with the retreating sea ice extent (Misund et al. 2016), climate-mediated community effects are also visible on lower trophic levels (Beaugrand 2003) towards a higher representation of Atlantic zooplankton species (Dalpadado et al. 2012). Thus, existing food web structures and thereby ecosystem functioning will alter by promoting the settlement of generalist predator species (Kortsch

et al. 2015). Hence, large boreal fish species, such as *G. morhua* and haddock (*Melanogrammus aeglefinus*) are becoming increasingly more common in the vicinity of Svalbard (Renaud et al. 2012), involving growing predation pressure on rather small Arctic fish species (Fossheim et al. 2015). Accordingly, the marine Artic species composition and thereby the existing Arctic food web is expected to experience tremendous changes within the near future. Finally, structural geographic borders as for instance the continental slope in the area North of Svalbard that marks the transition into the deep Arctic Basin may locally restrict the poleward migration of demersal species and support the local extinction of species depending on moderate habitat depths, while no such border exists for pelagic species (Hollowed et al. 2013).

The emphasis of this thesis is directed to two closely related (Møller et al. 2002) gadoid fish species inhabiting the Svalbard zone: the Arctic endemic cold-adapted Polar cod (*Boreogadus saida*), a highly abundant key species within the Arctic ecosystem and the cold-temperate Northeast Arctic population of the boreal Atlantic cod (NEAC) (*Gadus morhua*). The rising stock strength of *G. morhua* in the vicinity of Svalbard indicates that this species is in the process of becoming an increasingly important predator in the Arctic marine food web (Fossheim et al. 2015).

1.3.1 Polar cod (*Boreogadus saida*) as a model organism

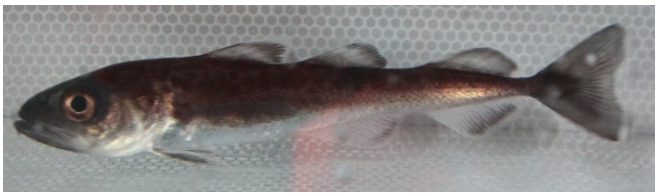

FIGURE 6. Polar cod (*Boreogadus saida*).

B. saida (FIG. 6) is a highly abundant gadoid species distributed throughout the Arctic. The Polar Front in the Barents Sea (Gjøsæter 2009), the fjords of Greenland (Christiansen et al. 2012) and cold waters off Northern Labrador and Newfoundland (Scott and Scott 1988) are marking its southernmost distribution in the North Atlantic. In the Barents Sea, *B. saida* was exploited by Norwegian and Russian fishing vessels mainly between 1966 and 1971 (Ajiad et al. 2011). Nowadays, *B. saida* is of marginal economic interest. Due to its

circumpolar distribution, its large standing stock biomass and lipid content, *B. saida* plays a key role in the Arctic food web by connecting higher and lower trophic levels (Hop and Gjøsæter 2013). Various species of whales, seals, birds and fish, including economically important fish species, primarily feed on *B. saida* (Sekerak 1982, Bradstreet et al. 1986, Welch et al. 1993). *B. saida* itself mainly preys upon ice-associated or pelagic copepods and amphipods as well as on more diverse epibenthic crustacean taxa later in life (Renaud et al. 2012). Accordingly, *B. saida* are found in a variety of habitats: Early life-stages and early juveniles of *B. saida* strongly depend on an under-ice habitat (Bradstreet 1982), whereas larger fish often occur pelagic or associated with the benthic habitat (Olsen 1962). Commonly, *B. saida* appear dispersed (Crawford and Jorgenson 1990) or in small aggregations (Lønne and Gulliksen 1989). Occasionally, however, *B. saida* are observed to form large schools (Ponomarenko 1968, Welch et al. 1993).

B. saida is a short-lived species with a maximum age of seven years. However, individuals older than five years are rarely found (Bradstreet et al. 1986). According to the short-lived lifestyle, male *B. saida* regularly mature at an age of two, while females become mature at an age of three years (Craig et al. 1982). Spawning of *B. saida* takes place from December to March (Hognestad 1968) with a peak spawning period in January and February (Rass 1968). Larvae appear until July, before they develop into juveniles at a body length of 30 – 50 mm in August (Rass 1968). During spawning, *B. saida* prefer a narrow thermal range above 0°C (1 – 2°C) (Hognestad 1966). Similar to spawners, pelagic 0-group *B. saida* avoid subzero habitat temperatures but are found in surface waters with a comparably broad temperature range (4 – 7°C Olsen 1962, 2 – 7°C Rass 1968, 0.3 – 5.2°C Falk-Petersen et al. 1986). Adult *B. saida* inhabiting Norwegian waters, in contrast, commonly prefer subzero temperatures (> -1.5°C), but occasionally appear up to 3.2°C (Falk-Petersen et al. 1986). Accordingly, *B. saida* from the Svalbard zone can experience temperatures between -1.5 – 7°C throughout its lifetime. *B. saida* from the Canadian Arctic, however, are documented to tolerate a slightly broader thermal range (-2 – 8°C) (Drost et al. 2014).

According to low habitat temperatures, *B. saida* generally show slow growth (Hop and Gjøsæter 2013), resulting in maximum body lengths of not more than 40 cm (Ajiad et al. 2011). Nevertheless, the assimilation efficiency is distinctly higher than for carnivorous fish in general and the efficiency to convert food into growth has been found to be even

higher compared to Antarctic fish (Hop et al. 1997) reflecting efficient mechanisms of cold-adaptation.

B. saida has been observed to be moderately active (Lønne and Gulliksen 1989) implying limited swimming capacities. Instead of fleeing, *B. saida* predominantly avoid predators by hiding in crevices underneath the ice cover (age 1 and 2, Lønne and Gulliksen 1989) or in benthic habitats in sill fjords that are characterized by subzero temperatures (Madsen et al. 2015).

1.3.2 Atlantic cod (*Gadus morhua*) as a model organism

FIGURE 7. Atlantic cod (*Gadus morhua*).

G. morhua (FIG. 7) is distributed throughout a broad latitudinal and thus thermal range (Brander 1994, 1995): In the Northeast Atlantic, *G. morhua* inhabits shelf seas from 45 – 80°N (Brander 2005), thereby facing habitat temperatures from -1 – > 20°C (Drinkwater 2005). Commonly, however, *G. morhua* is caught between 0 and 12°C (Drinkwater 2005).

G. morhua is sub-structured into several populations within the Atlantic Ocean (e.g. Mork et al. 1985, Pogson et al. 1995) with little gene flow across populations (Bradbury et al. 2014). In this thesis, I focused on the northernmost and largest population (Nakken 1994, Drinkwater 2009) of *G. morhua*, the cold eurytherm NEAC (hereafter termed as *G. morhua*) that represents the economically most important species in the Svalbard zone (Misund et al. 2016). *G. morhua* mainly inhabit offshore areas of the Barents Sea (Olsen et al. 2010), thereby experiencing temperatures between -1.5 and 11.7°C (Righton et al. 2010). Despite traditional intense fluctuations in stock size (Drinkwater 2009), a clear trend

for a northeastward distribution shift of *G. morhua* has been detected in the Svalbard zone in recent years, visible in an increasing abundance (Fossheim et al. 2015). While adult *G. morhua* were regularly caught in Svalbard waters (Woodhead and Woodhead 1959), year-round observations of juvenile specimens in coastal and fjord waters of Svalbard are only made since 2008 attributed to an increased volume transport of warm Atlantic water (Berge et al. 2015). This observation indicates that an overwintering stock of *G. morhua* is currently establishing in the vicinity of Svalbard.

G. morhua is a highly active top predator in the Barents Sea food web (Ajiad et al. 1992) with diets varying with body size (Nakken 1994): Copepods cover the largest part of the diet of larval *G. morhua*, replaced by euphausiids towards the end of the pelagic 0-group phase. Juveniles (< 50 cm) prey upon a wider variety of crustacean taxa (euphausiids, amphipods, shrimps), while other fish species (predominantly capelin, *Mallotus villosus*, and herring, *Clupea harengus*) and young congeners cover a substantial part of the diet of larger *G. morhua* (Nakken 1994). The diet of juvenile *G. morhua* inhabiting Svalbard waters has little overlap with the prey prioritized by co-occurring similar-sized *B. saida* (Renaud et al. 2012). Nevertheless, predation pressure on the comparably small *B. saida* is likely to rise due to a further expansion of adult *G. morhua* (Renaud et al. 2012, Fossheim et al. 2015).

Occasionally, *G. morhua* can reach a body length of 130 cm weighing up to 30 kg at age 20 (Olsen et al. 2010). Male and female *G. morhua* commonly reach maturity at an age of 6 – 7 and 7 – 8, respectively and undertake long migrations from the Barents Sea to their spawning areas along the Northwest coast of Norway. Spawning takes place between March and April (Olsen et al. 2010) at 4 – 6°C (Michalsen et al. 2014). Pelagic eggs are drifting in northeasterly-directed water currents into the Barents Sea. In late autumn, pelagic 0-group *G. morhua* change to a demersal lifestyle (Nakken 1994, Michalsen et al. 2014) and begin to search for prey both at the bottom and in midwater layers (Nakken 1994). Drinkwater (2005) discussed the possibility that future ice-free waters might cause *G. morhua* to cease spawning migrations and potentially even establish new spawning grounds as far north as Svalbard.

1.3.3 Mechanisms of cold-adaptation in Arctic fish species

Low thermal fluctuations throughout the past 0.7 – 2 million years (Eastman 1997) supported the adaptation of ectotherm species inhabiting the Arctic to the low temperatures faced in their habitat (Pörtner et al. 2000, Pörtner 2002a, b). Adaptations to low temperatures are manifested in the following mechanisms: In order to maintain fluid membranes at low habitat temperatures, cold-adapted species embed elevated proportions of unsaturated fatty acids into their membranes (compare review by Bell et al. 1986). Further, polar species possess elevated mitochondrial densities in red muscle tissue in order to compensate for depressed oxidative energy production in the cold (Dunn 1988). Moreover, polar ectotherms developed antifreeze glycoproteins that serve to bind on ice crystals in body fluids, thereby inhibiting their growth and preventing cell damage (DeVries et al. 1970). As a trade-off, these adaptations to low temperatures entail higher vulnerabilities of Arctic species to rapid physicochemical changes (Johnston 1990, Somero et al. 1996, compare review by Abele and Puntarulo 2004) compared to temperate species that developed to preserve performance throughout a wider thermal range comprising higher temperatures (Claireaux et al. 2006). Consequently, Arctic species at their southern distribution areas are expected to progressively succumb to competition from better-adapted invading temperate species during rising water temperatures.

1.4 Whole-animal energy budget – a tool to access climate-induced functional limitations

One suitable framework to access climate-induced organismic limitations and to predict the future fate of populations is the investigation of components of the whole-animal energy budget under environmental stressors (Sokolova 2013). The energy budget describes, how ingested energy (I) is allocated between aerobic processes of an organism as well as how much of the ingested energy is lost in terms of excretion (E) which covers both faeces and nitrogenous waste products. Energy demanding processes within the organism can be assigned to metabolism (M) and growth (G) (Brett and Groves 1979):

$$I = M + G + E$$

Metabolic costs are commonly approximated by oxygen consumption ($\dot{M}O_2$) measurements (Nelson 2016). $\dot{M}O_2$ measurements serve as an indirect calorimetric approach by representing the catabolic expenditure during the production of ATP (Nelson 2016).

Metabolism comprises standard, routine, active and feeding metabolism. Standard metabolism represents energetic baseline costs of resting, post-absorptive and, ideally, non-growing organisms (Chabot et al. 2016a). These baseline costs are predominately attributed to cellular maintenance, such as ion transport and the synthesis of biochemical constituents as well as respiratory and circulatory costs essential for the oxygen and nutrient supply of all body tissues (Jobling 1994). In contrast to standard metabolism, energetic demand for routine metabolism, active metabolism and feeding metabolism appears episodically (e.g. depending on the individual daily rhythm, predator encounters or the time after feeding) and add onto baseline metabolic costs. The active metabolic rate obtained by exhaustive exercise (experimentally achieved by critical swimming speed protocols or constant acceleration tests, see paragraph 2.5.4) (Norin and Clark 2016), is widely accepted as an estimate for one individual's maximum aerobic capacities (maximum metabolic rate) (Brett and Groves 1979). Accordingly, the difference between maximum metabolic rate (MMR) and standard metabolic rate (SMR) indicates the individual aerobic scope (AS) that is assumed to constitute the excess capacities available above maintenance for all aerobic activities at given environmental conditions (Fry 1971). Nevertheless, in some sluggish benthic ambush predators, the maximum metabolic rate evoked by digestion exceeds exercise-induced metabolic capacities by far (Clark et al. 2013). Accordingly, life-styles have to be considered for the assessment of metabolic capacities and their partitioning into aerobic processes. Further, exhaustive exercise involves the contribution of anaerobic metabolism and the mobilization of functional reserves entailing interpretation pitfalls (Pörtner et al. 2017).

Only when the requirements for baseline metabolism are met, surplus energy is allocated into growth (e.g. Wieser 1994, Sokolova 2013). Growth refers to somatic and gonadal growth and thereby reproduction (Jobling 1994). Somatic growth performance is directly related to individual fitness: Rapid growth reduces the time at vulnerable body size, thereby increasing chances for survival (Hunt von Herbing and White 2002). Further, most fish species first reproduce after reaching a certain body size (Kock and Kellermann 1991), and tissue energy reserves are essentially exploited during the development of reproduction

products (Jobling 1994). Energetic investment into reproduction is a precondition for natality, which determines – balanced by natural mortality – the abundance of a population or a species.

Energy allocation between aerobic processes of the whole-animal energy budget are strongly dependent on abiotic factors (e.g. Fry 1971, Pörtner and Farrell 2008, Sokolova 2013). Changes in abiotic conditions such as increasing water temperatures and OA modify aerobic capacities, resulting in energetic reallocations between the components of the energy budget, depending on the magnitude and rate of change (Pörtner et al. 2014). By integrating climate change-induced impacts on lower organizational levels (Pörtner 2002a, Pörtner et al. 2005), whole-animal performance under elevated temperatures and PCO_2 levels provides valuable insights for the evaluation of the respective populations' future competitive strength.

1.4.1 Effects of ocean warming on energy budgets

Within the species-specific thermal window, whole-animal performance supported by aerobic scope for activity increases with acute temperature rise reaching its optimum and decreases rapidly at temperatures approaching upper thermal limits (Pörtner and Farrell 2008, Pörtner et al. 2017). According to the concept of oxygen- and capacity limited thermal tolerance (OCLTT), decrements in aerobic performance at pejus ("getting worse") temperatures (T_{pej}) on both ends of the thermal window mark the onset of systemic oxygen limitations (Pörtner et al. 2017). Environmental temperatures beyond T_{pej}, therefore, entail detrimental effects on fitness-related functions such as growth, reproduction and activity as well as energy storage with implications on the population level. Functional constraints increase progressively towards upper and lower critical temperatures (T_{crit}). At T_{crit}, aerobic scope disappears (Pörtner 2002a), thereby solely allowing time-limited passive existence (e.g. Pörtner and Farrell 2008, Pörtner 2010).

Under acute warming conditions, a decrease in AS at temperatures approaching the upper T_{pej} is evoked by continuously exponentially increasing costs for baseline metabolism (e.g. Drost et al. 2016) that reflect increasing ATP turnover rates and a progressive increase in mitochondrial proton leakage with temperature (Pörtner et al. 2001), while maximum metabolic capacities are reaching a plateau (Pörtner 2010). Yet, most organisms have the capacity to compensate metabolic baseline costs to different degrees after chronic exposure

to new ambient temperatures by aid of acclimation processes (Precht 1958). Nevertheless, acclimation capacities to warming cues are species-specific and limited, and cannot fully prevent poleward shifts in distribution (Pörtner et al. 2017). Limitations in acclimation capacity and thereby thermal limitations evoked by a progressive mismatch in oxygen demand and oxygen delivery capacity beyond upper T_{pej} causes trade-offs in organismic energy budgets. Consequently, individual growth performance decreases which coincides with decreasing population growth, potentially resulting in a local decrease in field abundance. Likewise, the decreasing abundance of the stationary common eelpout (*Zoarces viviparus*) in its southernmost distribution area was attributed to environmental temperatures periodically surpassing this species' T_{pej} as identified in laboratory experiments (Pörtner and Knust 2007). In species relying on high sustained exercise performance, a reduction in AS in response to exceptionally high habitat temperatures has been shown to prevent successful spawning migrations with potential downstream effects on population recruitment (Farrell et al. 2008).

Further, thermal tolerance limits and thus the width of the species-specific thermal window differ throughout an organism's life-history with early life-stages and spawners being most vulnerable to thermal changes (Pörtner et al. 2008, Pörtner and Farrell 2008), thereby representing bottlenecks for population recruitment. Nevertheless, optimum temperatures commonly decrease with increasing body size (Björnsson et al. 2001). Accordingly, the abundance of large individuals of *Z. viviparus* in the German Bight was significantly stronger affected upon warming compared to smaller specimens (Pörtner and Knust 2007).

1.4.2 Effects of ocean acidification (OA) on energy budgets

Compared to invertebrates, marine teleosts are generally considered to be less sensitive towards moderately elevated PCO_2 levels as projected for the end of the century due to extensive acid-base regulation capacities (Melzner et al. 2009b, Wittmann and Pörtner 2013). Nevertheless, the costly regulation of OA-mediated acid-base disturbances can entail deleterious repercussions on metabolic performance (Strobel et al. 2013), osmoregulation (Heuer et al. 2012) and behavior (Nilsson et al. 2012), likely translating into detrimental effects on fitness (Heuer and Grosell 2014). The direction of responses of whole-animal parameters to elevated PCO_2 levels, however, is not uniform among marine teleost species (compare review by Esbaugh 2017). While energetic requirements for acid-base regulation are hypothesized to add onto baseline metabolic costs (loading stress)

(Pörtner 2010), elevated SMRs in response to moderate PCO_2 exposure are not consistently found throughout literature (bald notothen, *Pagothenia borchgrevinki*, at 4°C and dusky notothen, *Trematomus newnesi*, at -1°C, 28 days at ~ 1000 µatm, Enzor et al. 2013). Instead, SMR has been reported to be unaffected by near-future PCO_2 levels in several species (e.g. marbled rockcod, *Notothenia rossii*, 29 – 36 days at 2000 µatm, Strobel et al. 2012; emerald rockcod, *Trematomus bernacchii*, and striped rockcod, *Trematomus hansoni*, 28 days at ~ 1000 µatm, Enzor et al. 2013; red drum, *Sciaenops ocellatus*, 14 days at ~ 1100 µatm Esbaugh et al. 2016). Likewise, the response of elevated PCO_2 levels on the MMR and thereby AS is multidirectional and species-specific with species that experience highly fluctuating abiotic conditions generally being less sensitive to near-future PCO_2 conditions (Rummer et al. 2013b). Accordingly, the MMR of a variety of species was not impaired by chronic moderate PCO_2 exposure (*G. morhua*, 4 months at 3000 µatm, Melzner et al. 2009a; blue rockfish, *Sebastes mystinus*, 16 – 19 weeks at 750, 1900 and 2800 µatm, Hamilton et al. 2017). A reduced MMR under high PCO_2 conditions (limiting stress) (European sea bass, *Dicentrarchus labrax*, 67 – 69 days at 1000 µatm, Pope et al. 2014; copper rockfish, *Sebastes caurinus*, 14 – 17 weeks at 1900 µatm and 10°C, Hamilton et al. 2017), in turn, may potentially arise from an impaired oxygen affinity of hemoglobin (Esbaugh 2017) or reduced mitochondrial efficiencies (Strobel et al. 2013). A hypothesis proposed to explain elevated MMR in response to OA-exposure (Spiny damselfish, *Acanthochromis polyacanthus*, 17 days at 950 µatm, Rummer et al. 2013b) suspects plasma-accessible carbonic anhydrase located in highly aerobic tissue (Rummer and Brauner 2011) to support oxygen delivery to a greater extent under high compared to control PCO_2 conditions (Rummer et al. 2013a, Esbaugh 2017).

Species-specific varying trends in the response of aerobic scope to moderate hypercapnia suggest similar divergent downstream-effects of OA on individual performance parameters such as growth and exercise. In fact, growth performance that has largely been studied in early-life stages from varying latitudes was found to be negatively (anemonefish, *Amphiprion melanopus*, 31 days post hatching (dph) at ~ 1000 µatm, Miller et al. 2012; *C. harengus*, 15 – 17 dph at ~ 1800 µatm, Frommel et al. 2014) or positively influenced (flounder, *Paralichthys dentatus*, 28 dph at ~ 1800 µatm, Chambers et al. 2014; orange clownfish, *Amphiprion percula*, 11 dph at 550 – 1030 µatm, Munday et al. 2009) as well as unaffected (e.g. cobia, *Rachycentron canadum*, 22 dph at 800 and 2100 µatm, Bignami et al. 2013; walleye pollock, *Theragra chalcogramma*, ~ 30 dph at 280 – 2100 µatm, Hurst

et al. 2013; *D. labrax*, 45 dph at 1520 µatm, Crespel et al. 2017) during exposure to near-future PCO_2 conditions. Early-life stages, however, are considered to be more sensitive to hypercapnia due to their not fully developed acid-base regulation system (Melzner et al. 2009b). Despite recorded trends in larval organisms, available data agree on limited impacts of OA on fish growth (Esbaugh 2017), even at PCO_2 levels exceeding projected values for the year 2100 (> 1300 µatm) (Cattano et al. 2018).

Swimming performance was widely unaffected after long-term exposure to moderate PCO_2 conditions even independent of ontogenetic stage (e.g. larval *R. canadum*, 17 and 22 dph at 800 and 2100 µatm, Bignami et al. 2013; juvenile *G. morhua*, 4 months at 3000 µatm, Melzner et al. 2009a; juvenile *S. mystinus* 5 – 8 weeks at 750, 1900 and 2800 µatm, Hamilton et al. 2017). However, one study documented gradually reduced swimming performance after long-term exposure to different levels of elevated PCO_2 (juvenile *S. caurinus* 7 – 9 weeks at 750, 1900 and 2800 µatm, Hamilton et al. 2017).

Due to a more subtle impact of OA on physiological processes compared to warming effects, evidence of OA-induced limitations on teleosts in the field are rarely established (Pörtner et al. 2014). Interspecific contradictory responses of whole-animal performance parameters to OA and pre-adaptations to naturally high or strongly fluctuating PCO_2 levels further confound the detection of OA-induced effects in the ecosystem (Pörtner et al. 2014). Furthermore, climate drivers such as rising temperatures and OA are operating concurrently, thereby aggravating a clear attribution of biological observations to individual drivers (Parmesan et al. 2011). Evidences from a variety of marine taxa moreover suggest an enhanced sensitivity to OA at temperatures close to their upper thermal window (Pörtner 2012, Wittmann and Pörtner 2013), involving elevated energetic requirements for homeostasis (Cattano et al. 2018). Accordingly, acclimation capacities established for climate drivers investigated in isolation might be modified under realistic multiple-driver scenarios (Anttila et al. 2015). In order to access realistic ecological consequences of climate change on field populations, therefore, it is of utmost importance to expand knowledge about species- as well as life-stage-specific sensitivities towards multiple climate drivers (such as warming, OA, hypoxia).

1.5 Objectives and approaches

In light of the high rate of change of abiotic conditions projected for the Arctic Ocean, the present study focusses on acclimation capacities of two co-occurring fish species inhabiting waters in the vicinity of Svalbard, Norway, as part of the second phase of the German national program for OA research, BIOACID. More precisely, this study aims to access potential impacts of OAW scenarios on fitness parameters (such as growth and exercise performance) and associated constraining aerobic capacities of the endemic Arctic key species Polar cod (*Boreogadus saida*) and the invading boreal Atlantic cod (*Gadus morhua*) in order to predict their future competitive strength and potential changes in abundance.

A reasonable evaluation of consequences on an ecological level requires knowledge about long-term responses of species-specific whole-animal performance to realistic levels of multiple climate drivers. Further, it is vitally important to unravel acclimation capacities of less sensitive ontogenetic stages such as juveniles and non-spawning adults, containing genotypes that survived natural selection processes during vulnerable larval stages in order to gain a thorough picture of species resilience. Not least, juveniles are commonly inhabiting surface waters that are most susceptible to warming while being limited in their capacities to migrate to more favorable habitats.

Accordingly, I acclimated juvenile specimens of both *B. saida* and *G. morhua* to different elevated temperature and PCO_2 conditions based on end-of-the century scenarios projected by the IPCC for the determination of individual standard metabolism, growth performance and condition parameters (both species; publication I) as well as aerobic scope and swimming capacities (*B. saida*) (publication II). Further, the impact of concurrent elevated temperatures and food deprivation on aerobic capacities and swimming was investigated for juvenile *B. saida* (publication III).

In detail, the present thesis focused on the following objectives:

(i) As a first objective, I investigated the growth performance and associated metabolic costs of juvenile *B. saida* and *G. morhua* after chronic exposure to OAW scenarios, thereby identifying thermal limitations of both species at control and elevated PCO_2 levels. The findings for these routine performance parameters served to compare the future competitive strength of *B. saida* and *G. morhua* in the vicinity of Svalbard.

OBJECTIVE 1: DO ROUTINE WHOLE-ANIMAL PARAMETERS OF JUVENILE *B. SAIDA* AND *G. MORHUA* CHRONICALLY EXPOSED TO OAW SCENARIOS INDICATE LIMITATIONS OF SPECIES-SPECIFIC ACCLIMATION CAPACITIES?

(ii) Maximum performance capacities of juvenile *B. saida* acclimated to OAW scenarios were examined in order to assess potential impacts on energy utilization efficiencies and acclimation capacities, aiming to estimate this species' competitiveness and survival under future ocean conditions.

OBJECTIVE 2: WILL LONG-TERM OAW EXPOSURE AFFECT MAXIMUM PERFORMANCE CAPACITIES OF JUVENILE *B. SAIDA*?

(iii) In light of a decreasing prey availability for *B. saida* during ongoing ocean warming, I further investigated whether food deprivation compromised the sustainable swimming performance of *B. saida* acclimated to ambient and elevated temperatures, potentially causing modifications in metabolic prioritizations in future warming oceans.

In this context, the swimming performance and associated metabolic costs of *B. saida* were additionally assessed after acute exposure of cold- and warm-acclimated specimens to a common intermediate temperature in order to gain further insights about this species' aerobic acclimation capacities.

OBJECTIVE 3: DOES FOOD DEPRIVATION AFFECT MAXIMUM PERFORMANCE OF *B. SAIDA* AT DIFFERENT ACCLIMATION TEMPERATURES?

(iv) The contribution of anaerobic metabolism during critical swimming speed of *B. saida* was estimated based on its burst-and-coast swimming capacity at different OAW and feeding conditions. Further, the ecological significance of anaerobic swimming capacity for *B. saida* was discussed.

OBJECTIVE 4: HOW DOES BURST-AND-COAST SWIMMING PERFORMANCE OF JUVENILE *B. SAIDA* RESPOND TO FUTURE OCEAN WATER CONDITIONS? WHAT IS THE ROLE OF BURST-AND-COAST SWIMMING MODE FOR THIS SPECIES IN AN ECOLOGICAL CONTEXT?

2 Methods

All animal experiments were in accordance with the ethical standards of the federal state of Bremen, Germany. Holding conditions and handling were approved under the reference number 522-27-22/02-00 (113).

2.1 Sampling sites and catch method

For the present project, Polar cod (*Boreogadus saida*) were caught during two cruises (RV Helmer Hanssen, University of Tromsø, UiT, and RV Heincke, Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, AWI) in the vicinity of the Svalbard archipelago. Atlantic cod (*Gadus morhua*) were caught by RV Heincke in the same area (FIG. 8; for details, see TABLE 1).

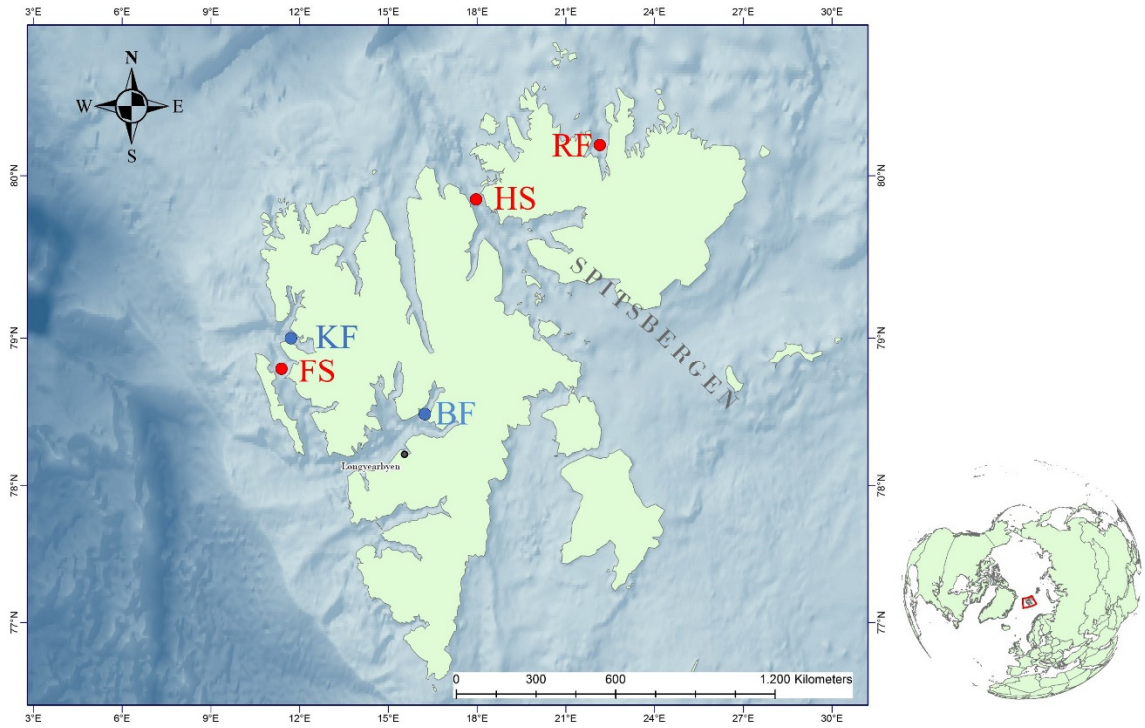

FIGURE 8. Sampling sites for *B. saida* (blue) and *G. morhua* (red). BF = Billefjorden, FS = Forlandsundet, HS = Hinlopenstretet, KF = Kongsfjorden, RF = Rijpfjorden.

TABLE 1: List and details of sampling sites.

Species	Vessel	Date	Location	Coordinates	Depth (m)	Temperature (°C)
B. saida	RV Helmer Hanssen	Jan. 2013	Kongsfjorden	78° 97' N 12° 51' E	220*	1.0 – 2.0
G. morhua	RV Heincke (HE 408)	Sept. 2013	Rijpfjorden	80° 15.42' N 22° 12.89' E	0 – 40	4.0 – 6.0
			Hinlopenstretet	79° 30.19' N 18° 57.51' E		
			Forlandsundet	78° 54.60' N 11° 3.66' E		
B. saida	RV Heincke (HE 451.1)	Sept. 2015	Kongsfjorden	78° 58.92' N 11° 45.99' E	194*	-1.7 – 0.0
			Billefjorden	78° 36.37' N 16° 30.74' E	150*	

* bottom layers.

All fish were caught by aid of a fish lift (Holst and McDonald 2000) connected to the cod-end of a bottom trawl (*B. saida*) or a pelagic trawl (*G. morhua*). The fish-lift is a device that size-selects for small individuals and provides a protected water body with little turbulence during the haul (FIG. 9). Hence, the fish-lift serves to reduce injuries and mortality rates during the catch of juvenile fish.

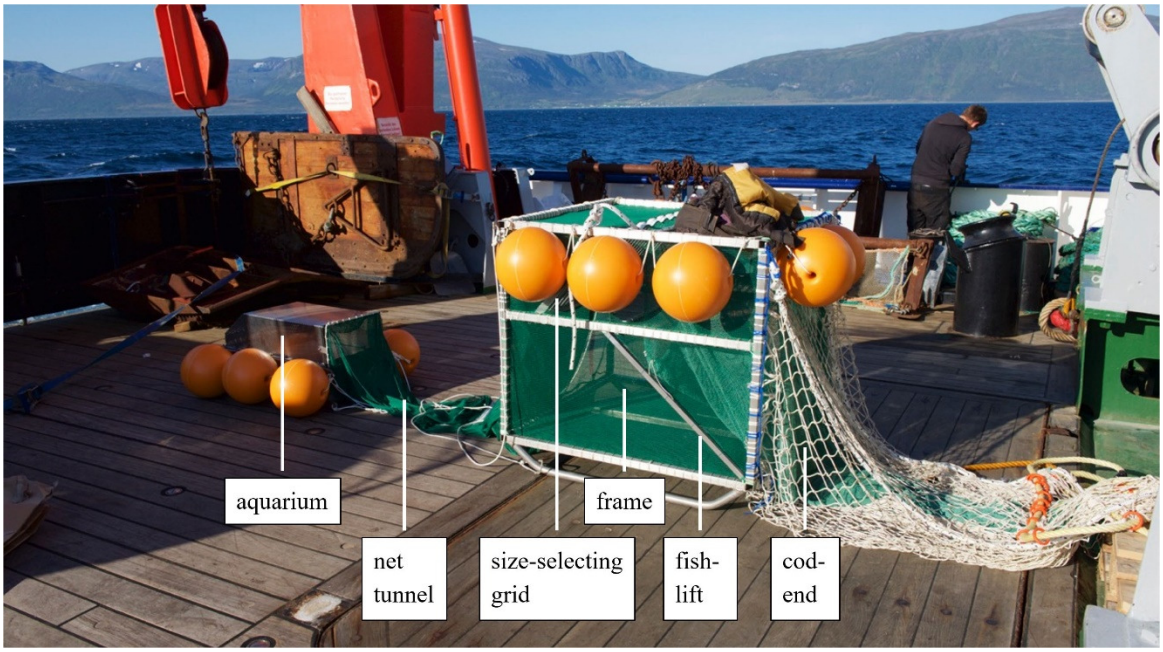

FIGURE 9. Fish-lift. Device for enhancing survival rates in juvenile fish during trawling. Picture by F. C. Mark.

2.2 Animal maintenance prior to incubation

Fish caught by RV Helmer Hanssen were kept at the Havbruksstasjonen i Tromsø AS (HiT) at 3.3 – 3.8°C for three months, before being transferred to the aquaria of the AWI in Bremerhaven. At HiT, the specimens were fed three times a week with frozen copepods (*Calanus spec.*). *B. saida* and *G. morhua* obtained from the RV Heincke were directly transported to the AWI in a thermostated recirculating aquarium system of 4 m^3. In the aquaria facilities of the AWI, fish were maintained in a recirculating water body (10 m^3) with water originating from the German Bight near Helgoland.

B. saida caught in January 2013 were weaned to the AWI aquaria for four weeks at 5°C prior to the start of the experiments. During this time of preconditioning, *B. saida* was fed daily with high protein food pellets (Amber Neptun, 5mm, Skretting AS, Norway). *G. morhua* was kept at the aquaria of the AWI for approximately seven months at 5°C prior to exposure to the experimental conditions. *G. morhua* was fed with a mixture of artificial food pellets (Amber Neptun) and natural food items (frozen copepods and baby krill) twice a week. *B. saida* caught in September 2015 were preconditioned for approx. two weeks at 1.5°C.

TABLE 2: Biological details of fish at the beginning of the experiment (mean values ± SEM).

Species	Date of catch	Start of incubation	Total length (cm)	Wet weight (g)	n
B. saida	Jan. 2013	May 2013	14.2 ± 0.2	17.2 ± 0.7	96
G. morhua	Sept. 2013	April 2014	18.0 ± 0.3	40.9 ± 1.9	96
B. saida	Sept. 2015	Oct. 2015*	12.4 ± 0.6	12.1 ± 0.2	25

* length and weight of *B. saida* was not determined at the beginning of the incubation, but prior to (feeding metabolism) or following the particular experiments (swimming performance).

2.3 Incubation at different temperature/PCO_2 scenarios

B. saida and *G. morhua* were incubated under different but stable temperature/PCO_2 conditions for 130 and 133 days, respectively.

2.3.1 Experimental conditions & incubation setup

Individuals of both species were slowly transferred to four different thermal conditions (maximum rate of temperature adjustment: 2°C d^{-1}) according to their thermal window (*B. saida*: 0, 3, 6, 8°C; *G. morhua*: 3, 8, 12, 16°C). Experimental temperatures were maintained by aid of thermostatted rooms. Each temperature was further combined with control and elevated PCO_2 conditions (390 and 1170 µatm, respectively). The low PCO_2 level reflected current habitat conditions. The elevated PCO_2 level represented the projected situation for the year 2100 based on the business-as-usual scenario concerning GHG emissions (RCP8.5) according to the IPCC (Pörtner et al. 2014). Hence, each incubation comprised eight different temperature/PCO_2 scenarios (FIG. 10).

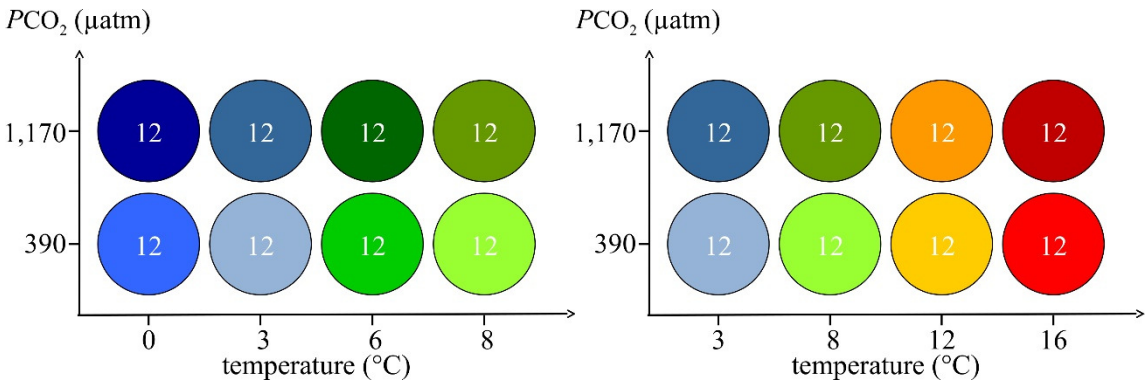

FIGURE 10. Experimental design for *B. saida* (left) and *G. morhua* (right). Numbers in circles represent n per treatment.

Animals were randomly distributed across experimental conditions in order to avoid a potential bias in biological replicates. Every treatment consisted of twelve single tanks (~ 24 L), each housing one individual (FIG. 11). The PCO_2 conditions were pre-installed in a treatment-specific header tank, which individually supplied the single aquaria (flow rate: ~ 500 mL min^{-1}).

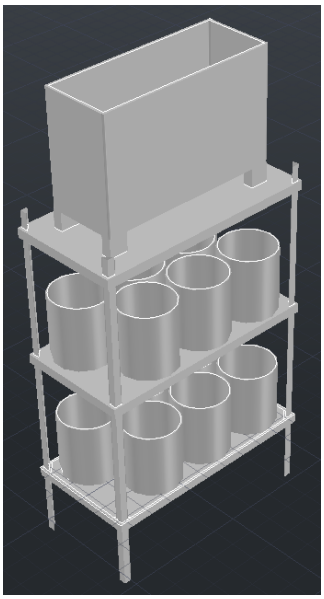

FIGURE 11. Experimental setup for long-term incubation. The picture shows one temperature/PCO_2 treatment (n = 12). Picture by S. Berger.

Every aquarium was cleansed daily from feces and other particles. Throughout the growth experiment a light rhythm of 12:12 h was installed with dimmed light conditions during day time.

2.3.2 Water chemistry

The PCO_2 conditions were generated by continuously aerating the water mass in the header tank with a mixture of virtually CO_2-free pressurized air and pure CO_2 installed with the aid of a mass flow controller (4 and 6 channel MFC system, HTK, Hamburg, Germany). For the verification of the PCO_2 conditions, pH, temperature and salinity were measured once to twice a week. During pH measurements in sea water, the pH meter (pH 3310, WTW, Weilheim, Germany) was calibrated with two thermally equilibrated NBS-buffers. The resulting pH values were cross-calibrated with Tris-buffered pH reference material (Batch 4, Marine Physical Laboratory, University of California, San Diego, USA) to total

pH scale in order to consider the ionic strength of sea water. Temperature and salinity were determined with a multimeter (LF 197 WTW, Weilheim, Germany).

Both pH_{tot} values and the concentrations of total dissolved inorganic carbon (DIC) that were determined by a Seal QuAAtro SFA Analyzer (800 TM, Seal Analytical, Mequon, USA) and verified in triplets were used to calculate sea water carbonate chemistry in the program CO2SYS (Lewis and Wallace 1998). For calculations in CO2SYS, the dissociation constants of Mehrbach et al. (1973) refitted by Dickson an Millero (1987) were applied, as well as the $KHSO_4$ constant after Dickson (1990). A full list of water chemistry raw data is given in PANGAEA (https://doi.pangaea.de/10.1594/PANGAEA.866369). Summary tables for water chemistry of both incubations (*B. saida* and *G. morhua*) can be found in publication I.

2.3.3 Water quality

A high water quality was maintained in the AWI aquaria (10 m^3) by aid of nitrification filters, protein skimmers, UV sterilizers and partial water exchanges. Due to space restrictions, single treatments (*B. saida*: 0 and 3°C, *G. morhua*: 12°C) had to be isolated from the recirculating water body. Each of the isolated treatments was provided with 1200 L of recirculating sea water. Water conditions were frequently monitored by aid of photometric NH_4^+ tests (Macherey-Nagel, Düren, Germany). A NH_4^+-value of 0.4 mg L^{-1} was accepted as threshold for partial water exchanges (~ 600 L). High energy turnover during the incubation with *G. morhua* required daily partial water exchanges combined with biological filter systems and protein skimmers (Sander, Germany) connected to each recirculating treatment. During the incubation of *G. morhua*, NH_4^+ (< 0.4 mg L^{-1}), NO_2^- (< 0.2 mg L^{-1}) and NO_3^- (< 50 mg L^{-1}) values were frequently recorded in the isolated treatments in order to continuously verify the filter function and to guarantee high water quality.

2.4 Incubation at different temperatures and feeding conditions

B. saida were incubated at 0 and 6°C and exercised under different feeding conditions at their respective acclimation temperature (71 and 45 days of acclimation before first swim trial at 0 and 6°C, respectively). At both acclimation temperatures, swimming performance and aerobic capacities were investigated in the same individuals in a fed and unfed stage.

Likewise, swimming performance and aerobic capacities were observed after acute exposure (2 h) of fed individuals to an intermediate temperature in order to evaluate potential acclimation capacities. Furthermore, the specific dynamic action (SDA) response of *B. saida* was characterized at 0 and 6°C after 106 and 12 days of acclimation, respectively.

2.4.1 Experimental conditions & incubation setup

The experimental temperatures reflected current habitat conditions (0°C) of *B. saida* as well as one elevated temperature (6°C) based on predicted mean surface temperatures for the Polar Arctic for 2081 – 2100 (relative to 1986 – 2005) according to the RCP6.0 (5.2 ± 1.9°C) (Collins et al. 2013). Further, 6°C represents the peak temperature for the aerobic scope of *B. saida* under *ad libitum* food conditions as observed in previous experiments (publication II).

At both temperatures, two groups of *B. saida* (n = 8, n = 5) were kept in two separate aquaria (~ 45 L each). Both aquaria were supplied by a common pre-conditioned water body of approximately 1000 L. Individuals of the larger groups were used for swimming performance experiments (FIG. 15). SDA experiments were performed with individuals from the smaller group (FIG. 16).

2.4.2 Feeding

B. saida were fed daily with baby krill (*Euphausia pacifica*) (Erdman, Ritterhude, Germany) within the first six weeks of incubation. Subsequently, the feeding schedule changed from daily feeding events to three feeding events per week. However, four days prior to SDA or exercise experiments, a daily feeding routine was established.

Instead of force-feeding, animals were fed in groups in order to stimulate individual food intake due to a general feeding activity (Hop and Tonn 1998). In addition, this procedure served to minimize handling stress. All fish were allowed to feed in the dark from a determined amount of krill for precisely two hours, before being transferred to the swim tunnel or to the respiration chamber (for SDA experiments). In case of swimming experiments, the food remained in the common tank until the first swim trial was completed. Immediately after placing the second fish in the swim tunnel (two swimming trials per day; see chapter 2.5.4.2), remaining food items were removed from the common tank and weighed for the determination of the amount of consumed food per treatment.

For the duration of food deprivation, see the paragraphs 2.5.4.2 (constant acceleration test) and 2.5.5.4 (specific dynamic action).

2.4.3 Water quality

Water temperatures and NH_4^+ levels were verified twice a week by aid of a multimeter (WTW LF 197, WTW, Weilheim, Germany) and photometric test kits (Macherey-Nagel, Düren, Germany), respectively. The water was partially exchanged (~ 600 L) when NH_4^+ levels ≥ 0.4 mg L^{-1} were recorded.

2.5 Whole-animal parameters

A list of raw data for individual whole-animal parameters of *B. saida* and *G. morhua* at rest obtained after long-term exposure to different temperature/PCO_2 conditions (publication I) can be found in PANGAEA (https://doi.pangaea.de/10.1594/PANGAEA.867390; for mean values per treatment, see TABLE A1. Raw data on individual maximum performance parameters of *B. saida* acclimated to different temperature/PCO_2 levels (publication II) can be found in PANGAEA (https://doi.pangaea.de/10.1594/PANGAEA.889447; for mean values per treatment, see TABLE A2) as well. Likewise, raw data on aerobic capacities and swimming performance in fed and unfed *B. saida* (publication III) are published in PANGAEA (https://doi.pangaea.de/10.1594/PANGAEA.889161; for mean values per treatment, see TABLE A3).

An overview of all fitness-related whole-animal parameters recorded in the framework of this PhD project is given in TABLE 3.

TABLE 3: List of recorded and calculated whole-animal parameters.

Species	Parameter	Abbreviation	Unit	n per publication*,**
Boreogadus saida & *Gadus morhua*	mortality		%	I: 96; 96
	specific growth rate	SGR	wt. % d^{-1}	I: 94; 85
	daily food consumption	F	g BW^{-1} d^{-1}	I: 94; 85
	food conversion efficiency	FCE		I: 94; 85
	condition factor	CF		I: 94; 85
	hepatosomatic index	HSI	%	I: 86; 66
	gonadosomatic index	GSI	%	I: 71 male, 13 fem.; 20 male, 40 fem.
	standard metabolic rate	SMR	µmol O_2 min^{-1} g^{-1}	I: 42; 70 II: 40 III: 10; 15; -; -
	stomach filling	SF	% stomach wt.	I: 80; 52
Boreogadus saida	maximum metabolic rate	MMR; MMR_{dig}; MMR_{ex}; MMR_{ex+dig}	µmol O_2 min^{-1} g^{-1}	II: 38 III: 7; 12; 14; 14
	aerobic scope	AS; AS_{dig}; AS_{ex}; AS_{ex+dig}	µmol O_2 min^{-1} g^{-1}	II: 35 III: 7; 12; 14; -
	gait transition speed	U_{gait}	BL sec^{-1}	II: 37 III: -; 11; 13; 14
	critical swimming speed	U_{crit}	BL sec^{-1}	II: 38 III: -; 11; 13; 14
	swimming efficiency	E_{max}	BL g μmol^{-1}	II: 34 III: -; 12; 13; 14
	maximum burst count	BC_{max}		II: 42 III: -; 12; 13; 14
	total no. of bursts	BC_{tot}		II: 42
	time betw. U_{gait} & U_{crit}***	TSB	sec	II: 42
	proportion of anaerobic metabolism****	$TSB_{anaerob}$	%	II: 42
	SDA duration	SDA duration	h	III: 7
	SDA magnitude	SDA magnitude	µmol O_2 g^{-1}	III: 7

* publication I: n for *B. saida*; n for *G. morhua*; ** publication III: n for fed, unexercised treatment; n for unfed, exercised treatment; n for fed, exercised treatment; n for fed, exercised fish acutely exposed to 3°C; *** "time spent bursting"; **** putative proportion of anaerobic metabolism during the period between U_{gait} and U_{crit} with an estimated duration of one second per burst; wt. = weight; BW = body weight; d = day; BL = body length.

2.5.1 Specific growth rate

Every specimen was weighed (wet weight; to the nearest 0.1 g) and measured (total length; to the mm below) the third day after feeding at three stages during the incubation: i) Before transfer into the incubation setup, ii) after approximately half-time of the incubation period (*B. saida*: day 60 – 68; *G. morhua*: day 44 – 47) and iii) in combination with respiration experiments that represented the end of the incubation period (*B. saida*: day 109 – 130; *G. morhua*: day 108 – 133). During the first two measurement events, a mild sedation (0.06 g L^{-1} MS-222) was used to enhance the accuracy of the measurements. Further, the sedation helped to accelerate the measuring procedure and thereby to reduce the time of air exposure. The obtained weight data from measurement events one and three served to calculate the specific growth rate (SGR) per day according to Jobling (1988):

$$SGR = 100 \frac{(\ln W_{end} - \ln W_{start})}{t_{end} - t_{start}},$$

with W_{start} and W_{end} representing the individual wet weight in gram at the first (t_{start}) and the respective last day (t_{end}) of incubation.

2.5.2 Food consumption & food conversion efficiency

During the growth experiment, fish were fed *ad libitum* every fourth day with high-protein food pellets (Amber Neptun, 5 mm, Skretting AS, Norway) (for composition, see TABLE 4).

TABLE 4: Relative nutrient composition of artificial food.

Component	Amount (%)
Protein	54.7
Fat	19.1
Carbohydrates	8.0
Moisture	8.5

Fish were allowed to feed on a determined amount for several hours, before the remaining food items were removed, dried (24 h at 80°C) and weighed again for the determination of

individual food uptake. For a better acceptance of artificial food during the incubation with *B. saida*, food pellets were soaked with a determined amount of sea water for 24 h after weighing.

Preliminary investigations revealed a slightly different humidity level (hl) between food pellets prior to handling and food pellets after drying for 24 h at 80°C. Accordingly, a correction factor (hl) (*B. saida*: 0.8820, *G. morhua*: 1.0227) was considered during the calculation of individual food uptake per meal (F_{meal}):

$$F_{meal} = hl\, F_{in} - F_{out},$$

with F_{in} and F_{out} representing the food weight per individual meal and the weight of remaining food items, respectively. For the daily food consumption (F), the resulting value was divided by four according to the feeding frequency. Finally, the daily food consumption was presented per body weight by using the individual weight data from the midterm measurement.

Further, weight gain and food uptake (in gram) were used to calculate the individual food conversion efficiency (FCE):

$$FCE = \frac{(W_{end} - W_{start})}{F}.$$

For the determination of exact ratios during the incubation at different temperatures and feeding status, baby krill was carefully patted dry with paper towels before weighing. For details about the feeding schedule, see paragraph 2.4.2.

2.5.3 Body index parameter (CF, HSI, GSI)

In order to predict the fitness of *B. saida* and *G. morhua* under the different abiotic conditions, a set of condition factors was investigated. The body condition factor (CF) was calculated according to Fulton (1911):

$$CF = 100\, \frac{W}{L_t^{\,3}},$$

with W representing the wet weight in gram and L_t representing the total length in centimeter.

The liver wet weight (W_L) was used to calculate the hepatosomatic index (HSI):

$$\mathrm{HSI} = 100\ \frac{W_L}{W}.$$

Further, gonad development was estimated by aid of the gonadosomatic index (GSI):

$$\mathrm{GSI} = 100\ \frac{W_G}{W},$$

with W_G being the gonad wet weight in gram.

2.5.4 Swimming performance

The swimming performance of *B. saida* was investigated in a Brett-type swim tunnel (Loligo Systems ApS, Denmark). A combination of low water temperature and little biomass, however, required that the MMR was recorded as excess post exercise oxygen consumption (EPOC) in a separate setup comprising respiration chambers (see paragraph 2.5.5).

The swim chamber of the Brett-type swim tunnel was submerged in a reservoir tank (together ~ 30 L), that supported stable abiotic conditions within the chamber. A separate zone within the swim chamber served as working section (46.5 x 13.5 x 14 cm) for the fish. The water velocity in the swim chamber can be governed with the aid of a control unit. The control unit regulated the engine that was connected to a propeller within the swim chamber. A honeycomb grid upstream of the working section ensured a uniform water velocity profile and a laminar flow (FIG. 12). A close-up view of the working section is provided in FIG. 13.

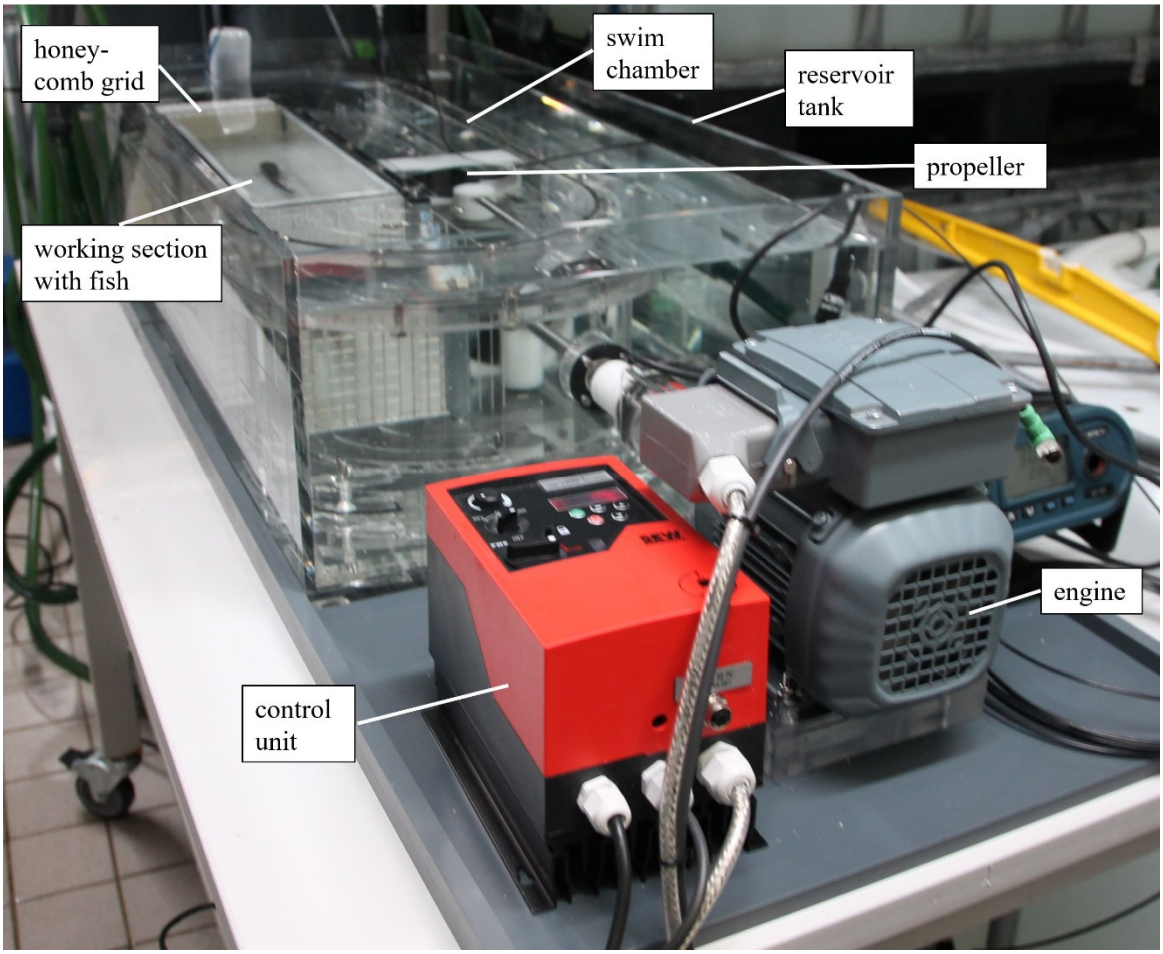

FIGURE 12. Swim tunnel with *B. saida* in working section.

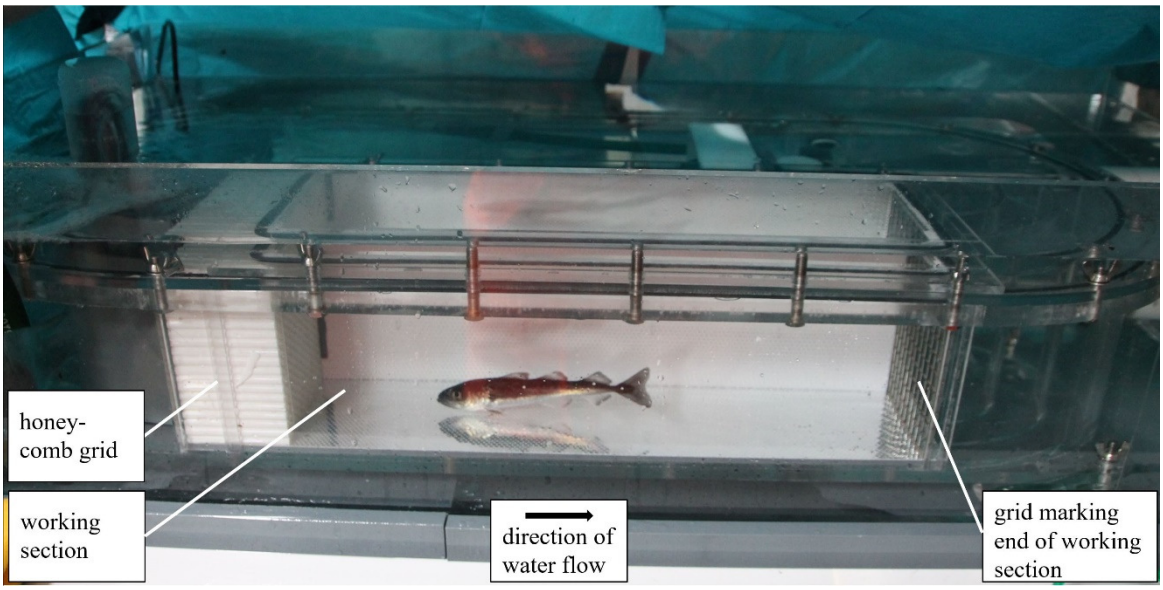

FIGURE 13. Close-up view of working section with *B. saida*.

In order to translate voltage output into water velocity (cm sec^{-1}), the velocity of the water current was measured with a flow sensor (Vane wheel flow sensor, FA, Höntzsch Instruments, Waiblingen, Germany) in the working section throughout a wide range of voltage output. This calibration was performed before and after each set of experiments (FIG. 14).

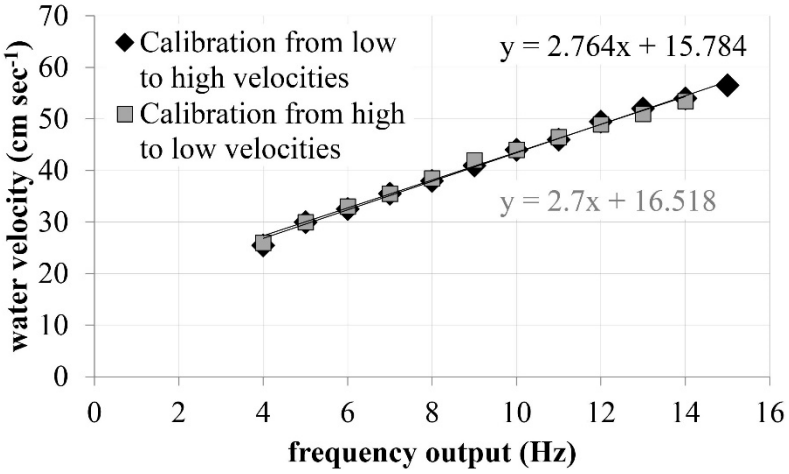

FIGURE 14. Example of calibration line of swim tunnel. The formula of trend lines were used to calculate intermediate swimming speeds.

During settling periods and swim trials, the tunnel was covered with an opaque plastic curtain in order to minimize external disturbances.

In the present PhD project, two different swimming protocols were applied in order to investigate different aspects of the swimming performance of *B. saida*: At the end of the temperature/PCO_2 incubation, a critical swimming speed (U_{crit}) protocol was performed in order to reveal potential impairments of ocean acidification and warming (OAW) scenarios on the predominantly sustained swimming performance of *B. saida*. A variation of the U_{crit} protocol, closely related to a constant acceleration test (CAT) (hereafter referred to as CAT protocol), was applied for *B. saida* of the temperature/feeding condition experiment aiming at identifying metabolic prioritizations between digesting and swimming. This second approach to measure swimming performance mainly differs from a classic U_{crit} protocol by distinctly shorter durations at single velocity steps (ideally: steady velocity increase). In CAT protocols, therefore, exhaustion occurs at higher velocities and within a shorter time frame compared to U_{crit} protocols (Reidy et al. 1995, 2000). The continuous increase in

velocity and the concomitantly higher drag causes a stronger recruitment of anaerobic metabolism in CAT protocols, while anaerobic energy during U_{crit} protocols is only recruited briefly before exhaustion (Reidy et al. 1995). Both protocols are described in detail in the following paragraphs.

2.5.4.1 Critical swimming speed protocol

Following the incubation to different temperature/PCO_2 scenarios, the (endurance) swimming performance of *B. saida* (n = 4 – 6 per treatment) was investigated in a critical swimming speed (U_{crit}) protocol.

The swim tunnel was filled with pre-conditioned water from the respective treatments. To maintain abiotic water conditions throughout the experiment, the swim tunnel was placed in temperature-stable rooms and the reservoir tank was constantly aerated with the CO_2/air mixture according to the acclimation conditions of the respective individuals. Throughout the whole protocol, the swim chamber was supplied with water from the surrounding reservoir tank by aid of an aquarium pump (9.2 L min^{-1}) in order to prevent decreasing oxygen concentrations or fluctuating temperature/PCO_2 conditions.

The experiment started the third day after feeding. After a settling period at a low water speed (1.4 – 2.2 BL sec^{-1}), the velocity was continuously increased to a starting velocity of 2.4 – 2.8 BL sec^{-1} with similar relative start velocities depending on the size of the fish. From the starting velocity on, every velocity step was maintained for 10.5 min, before the speed was carefully increased by 1.9 ± 0.3 cm sec^{-1} within 30 sec. As soon as the locomotion of the fish transitioned from steady cruising to occasional burst-and-coast events, the individual gait transition velocity (U_{gait}) was reached. U_{gait} marks the velocity at which the individual begins to partly fuel the rising metabolic demand for swimming by aid of anaerobic metabolism (Peake and Farrell 2004). In the present project, however, U_{gait} is interpreted as the maximum sustainable swimming speed. The U_{crit} protocol ended and the velocity was quickly reduced when the fish's tailfin touched the grid at the end of the working section for at least 30 sec. Immediately afterwards, the individual was removed from the swim tunnel and transferred into a respiration chamber. U_{crit} was adjusted according to Brett (1964) integrating the individual time spent at the highest velocity step:

$$U_{crit} = V + \frac{T}{t} \upsilon ,$$

with V = highest speed maintained for full time interval, T = amount of time spent at fatigue velocity in minutes, t = time interval (11 min), υ = velocity increment.

The efficiency of maximum swimming performance (E_{max}) was assessed according to the formula:

$$E_{max} = \frac{U_{crit}}{MMR_{ex}},$$

with MMR_{ex} representing the maximum metabolic rate of unfed *B. saida* at U_{crit}.

2.5.4.2 Constant acceleration test

The impact of food deprivation on the swimming performance of *B. saida* at 0 and 6°C (n = 7 and n = 8, respectively) was investigated in a protocol closely related to a CAT approach (for details concerning the difference to a traditional CAT protocol, see end of the present paragraph). Further, acclimation capacities of *B. saida* acclimated to 0 and 6°C were assessed by performing swimming tests on fed fish acutely exposed to 3°C.

Fish were allowed to voluntarily feed for at least two hours in their common holding tank before being transferred into the swim tunnel. *B. saida* that were supposed to swim at acute exposure to 3°C were also fed in the common tank at their respective acclimation temperatures prior to exercise experiments. For details concerning the feeding procedure, see paragraph 2.4.2. For the investigation of swimming performance in unfed individuals, feeding was intermitted for 18 – 21 days and for 10 – 13 days at 0 and 6°C (FIG. 15).

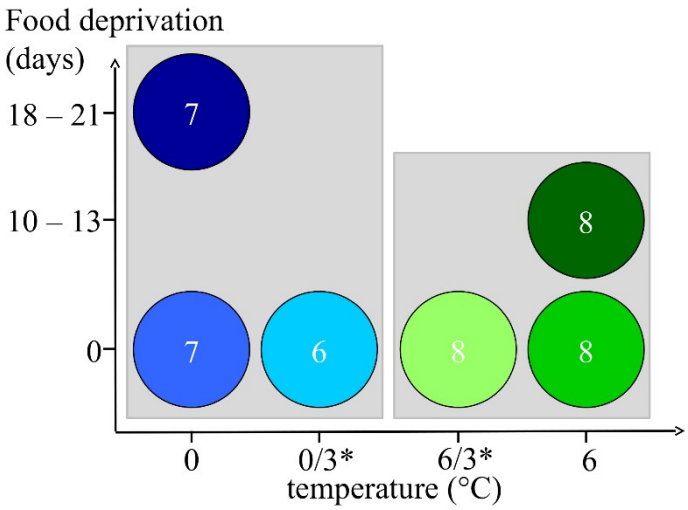

FIGURE 15. Experimental design for *B. saida* during swimming performance experiments following feeding and temperature-specific food deprivation periods. Numbers in circles represent n per treatment. Grey shadings optically combine the same specimens of *B. saida* measured at three different conditions (fed at acclimation temperature, food-deprived at acclimation temperature, fed before acute exposure to 3°C). * = acclimation temperature/(acute) experimental temperature.

In the swim tunnel, all fish were allowed to recover from handling stress (transfer holding tank – swim tunnel) without water current for exactly two hours. Accordingly, the period of the exercise protocol fell into the time frame of maximum metabolic rate in response to digestion (MMR_{dig}) in non-exercising fish.

The experimental setup for the CAT closely resembled the one of the U_{crit} test: Aeration took place continuously in the reservoir tank surrounding the swim chamber, which was filled with preconditioned water. Throughout the CAT, the swim tunnel was in an open mode with an aquarium pump (9.2 L min^{-1}) ensuring a permanent water exchange between reservoir tank and swim chamber and thereby high oxygen concentrations within the swim chamber. However, one essential modification was undertaken in the setup of the CAT protocol: Observations during preliminary experiments revealed that the smaller body length of specimens used in the CAT compared to the U_{crit} protocol (compare TABLE 5) allowed the fish to outlast the acceleration of the water body by hiding in an angle of the working section with reduced water velocity. Therefore, the width of this angle was diminished by inserting a second honeycomb device into the working section. The concomitant reduction of the working section length (from 46.5 to 33.5 cm) did not reveal signs of hampered swimming performance in any of the investigated individuals.

After two hours of acclimation to the swim tunnel, the CAT protocol started with a settling period of 5 min at 26 cm sec^{-1}. Following this settling period at a low velocity, the water current was accelerated by 1.4 cm sec^{-1} within ~ 10 sec and kept stable for 50 sec for the quantification of burst-type swimming events that are considered as a measure for anaerobic swimming in the present PhD project (see paragraph 2.5.4.3). By briefly interrupting the acceleration, the CAT applied in this thesis differed from a traditional CAT protocol. Velocity increments were repeated until the fish reached U_{crit}, marked by physical contact to the grid for 30 sec. As soon as U_{crit} was reached, water speed was rapidly reduced and fish were transferred into separate respiration chambers. This protocol allowed the accomplishment of two swimming trials per day.

E_{max} was calculated according to the formula given in paragraph 2.5.4.1, using MMR_{ex} for unfed and MMR_{ex+dig} for fed fish.

2.5.4.3 Anaerobic swimming performance

In the present thesis, the anaerobic swimming performance of *B. saida* was investigated by counting burst-and-coast swimming events that indicated white muscle contractions (for details, see paragraph 4.3.2):

During the U_{crit} protocol, burst counts were performed at every velocity step after 5 and 10 min and lasted for exactly 30 sec. The mean of both burst records served as the individual burst count per velocity. During the CAT, burst-type swimming events were counted for 50 sec at every velocity step.

For the estimation of anaerobic swimming capacities, both the maximum burst count per velocity step (BC_{max}) as well as the total burst performance throughout the U_{crit} protocol were considered. The latter represented the putative contribution of anaerobic metabolism (%) to the swimming performance above U_{gait}, when approximating a duration of one second per burst:

$$TSB_{anaerob} = BC_{tot} \frac{100}{TSB},$$

with TSB = time spent bursting (time between U_{gait} and U_{crit}) (sec), BC_{tot} = total number of bursts, $TSB_{anaerob}$ = estimated proportion of anaerobic metabolism between U_{gait} and U_{crit}.

2.5.5 Respiration measurements

In the present study, oxygen consumption ($\dot{M}O_2$) was considered as a proxy for metabolic rates as outlined by Nelson (2016). The highest $\dot{M}O_2$ in response to exhaustive exercise is considered as the maximum metabolic rate (MMR), while $\dot{M}O_2$ in resting fish reflects the standard metabolic rate (SMR). $\dot{M}O_2$ measurements were performed with the aid of automated intermittent-flow respirometry subsequent to swimming performance experiments with two exceptions: The SMR of *G. morhua* was recorded without previous exhaustive exercise. Likewise, the specific dynamic action (SDA) of *B. saida* following acclimation to 0 and 6°C was obtained in previously resting fish.

Fish were inserted into respiration chambers the third day after feeding (publication I & II), the day of feeding or following a food deprivation period (publication III) (TABLE 5).

The wet weight of each individual was quickly recorded during the transfer between swim tunnel and respiration chamber. The total length, in turn, was determined upon completion of respiration experiments in order to minimize the period of air exposure. Biological data of fish used in respiration measurements are presented as mean values in TABLE 5.

TABLE 5: Biological details (mean values ± SEM) of *B. saida* during respiration measurements.

Incubation	Swimming protocol	Species	Total length (cm)*	Wet weight (g)*	Days after feeding**	n
temperature/PCO_2	U_{crit}	*B. saida*	15.5 ± 0.2	26.1 ± 1.3	3	41
temperature/PCO_2	-	*G. morhua*	22.1 ± 0.3	89.0 ± 4.7	3	70
temperature/feeding	CAT	*B. saida*	13.0 ± 0.1	14.8 ± 0.5	0; 18 – 21 (0°C), 10 – 13 (6°C)	43***
temperature/feeding	- ****	*B. saida*	11.3 ± 0.3	10.1 ± 0.9	18 (0°C), 9 (6°C)	10

* for more detailed values according to temperature/PCO_2 or temperature/feeding treatments see publication II and publication III, respectively; ** days after feeding correspond to the first day of respiration experiments; *** three replicates per individual (unfed and fed at acclimation temperature, fed at one intermediate temperature). For number per treatment compare publication III; **** *B. saida* used for SDA investigations.

For the estimation of individual $\dot{M}O_2$, perspex respiration chambers (1.8 and 2.2 L) were submerged into a water basin (~ 50 L) with abiotic conditions identical to the respective acclimation conditions (except for fed fish acutely exposed to 3°C; publication III).

Aeration took place in the water surrounding the respiration chambers with compressed air or the same compressed air/$P\mathrm{CO_2}$ mixture as applied during the acclimation period, respectively. Further, air stones were submerged in a tube in a corner of the water basin in order to prevent small diffusive air bubbles from being pumped into the respiration chambers. Partial water exchanges with preconditioned water were performed once a day with the respiration chambers remaining fully covered with water at any given moment. Apart from water changes, aquaria containing the respiration chambers were covered with an opaque lid in order to reduce external disturbance.

All respiration chambers were equipped with a recirculation pump (8.2 L $\mathrm{min^{-1}}$) and a flush pump (5.0 L $\mathrm{min^{-1}}$). The recirculation pump created a slow constant current through the chamber and the connected tubing system to warrant correct $P\mathrm{O_2}$ conditions at the oxygen probes. With the aid of a flush pump, a periodic water exchange was accomplished between the respiration chamber and the surrounding water tank after each measurement cycle (for duration of measurement periods, see publication I – III), thereby replenishing oxygen levels within the chamber. The periodic decrease in oxygen concentration was used to calculate $\dot{M}\mathrm{O_2}$.

Oxygen concentrations were recorded using various types of oxygen-meters (ten channel oxygen-meter, PreSens-Precision Sensing GmbH, Regensburg, Germany; Fibox 3 system, PreSens-Precision Sensing GmbH; FireStingO2, Pyro Science GmbH, Aachen, Germany) and determined by optical oxygen probes. The 0 %-calibration was performed once at room temperature by flushing the oxygen probes with $\mathrm{N_2}$ until stable values were recorded. The 100 %-calibration of the oxygen probes, in turn, was performed prior to every trial within fully aerated water at the respective experimental temperature. Blank measurements to detect bacterial respiration were performed once at every temperature (temperature/$P\mathrm{CO_2}$ incubation) or subsequent to respiration experiments of each trial (temperature/feeding condition incubation) for several hours or in one spare respiration chamber simultaneous to SDA experiments.

Individual $\dot{M}\mathrm{O_2}$ values in the present PhD study were corrected for bacterial respiration and calculated using the following equation:

$$\dot{\mathrm{M}}\mathrm{O_2} = \frac{c\mathrm{O_2} * \mathrm{V} * \Delta P\mathrm{O_2}}{\mathrm{BW}},$$

with $\dot{M}\mathrm{O_2}$ = oxygen consumption rate (µmol $\mathrm{min^{-1}}$ $\mathrm{g^{-1}}$)

cO_2 = αO_2 * (($P_{air} - P_{water}$) * 0.2095))

Oxygen concentration during full oxygen saturation (µmol L^{-1}) with αO_2 (µmol L^{-1} $torr^{-1}$) = temperature- and salinity-dependent oxygen solubility in water, P_{air} (torr) = partial pressure of oxygen and P_{water} (torr) = temperature-dependent water vapor pressure (Boutilier et al. 1984).

V = water volume in respiration chamber and tubing excluding the respective fish volume (L)

ΔPO_2 = decrease in oxygen partial pressure per minute due to respiration

BW = wet body weight (g)

Further, $\dot{M}O_2$ values were adjusted to the average fish weight ($BW_{average}$) in publication II (25.6 g) and III (14.0 g) according to the equation of Steffensen et al. (1994):

$$\dot{M}O_{2\,(BW_{average})} = \dot{M}O_2 \left(\frac{BW}{BW_{average}}\right)^{(1-0.8)}$$

2.5.5.1 Maximum metabolic rate

In the present PhD study, the MMR evoked by exercise was determined as EPOC subsequent to an U_{crit} or CAT protocol (Bushnell et al. 1994, Marras et al. 2010). In publication II, solely the initial five minutes of the first $\dot{M}O_2$ record were considered for the calculation of the MMR, while the entire first $\dot{M}O_2$ record lasting for 60 minutes served to determine the MMR in publication III.

2.5.5.2 Standard metabolic rate

In the respiration chambers, fish were allowed to recover from exercise and handling stress for at least 48 h. The corresponding individual SMR was calculated by aid of three different approaches (Chabot et al. 2016a):

- The average of the five lowest, consecutive $\dot{M}O_2$ values (publication I)
- The 15 % quantile among $\dot{M}O_2$ values starting from the second night in the respiration chambers (publication II). A quantile approach has been considered as a reliable technique in the determination of SMR due to a strong temporal variability in this variable (Chabot et al. 2016a).

- The 20 % quantile among $\dot{M}O_2$ values starting 24 h after placement in the respiration chambers (swim trial) or among $\dot{M}O_2$ values obtained the 18th (0°C) or 9th day (6°C) subsequent to the termination of the SDA response (publication III).

In publication III (third approach), only unfed fish were considered for the determination of SMR following swim trials.

2.5.5.3 Absolute aerobic scope

The aerobic scope (AS) was calculated according to the equation:

$$\mathrm{AS} = \mathrm{MMR} - \mathrm{SMR}$$

2.5.5.4 Specific dynamic action

After daily feeding for four days, the SDA response of *B. saida* was recorded for 18 (0°C, n = 5) and 9 days (6°C, n = 3) (FIG. 16). This rather long period was chosen due to slow gastric evacuation rates of *B. saida* (evacuation half-time: 146 h, -0.49°C, Hop and Tonn 1998) at low water temperatures. In the present study, fish were allowed to feed on baby krill (consumed amount: 3.5 % BW, 0°C; 3.6 % BW, 6°C) for two hours prior to transfer into the respiration chambers.

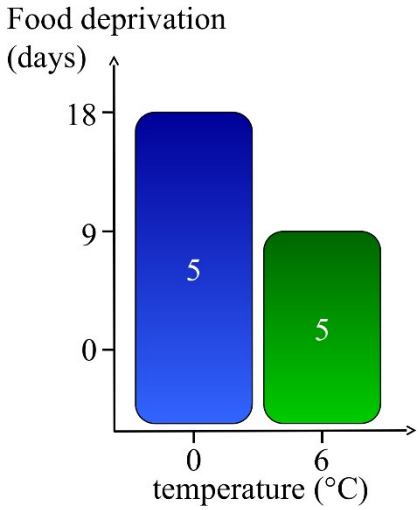

FIGURE 16. Experimental design for SDA experiments with *B. saida*. Numbers in rectangles represent n per treatment.

The setup for SDA experiments as well as the calibration of oxygen probes, the maintenance and consideration of bacterial respiration measurements were identical as described above (paragraph 2.5.5). Further, water temperature was continuously recorded. High water quality during the SDA measurement was maintained by daily partial water exchanges and verified periodically by measuring NH_4^+ concentrations.

Individual SDA responses were characterized by the following variables: (1) absolute peak $\dot{M}O_2$ (MMR_{dig}), (2) SMR and (3) aerobic scope for digestion (AS_{dig}), as well as (4) SDA duration and (5) magnitude (FIG. 17). For the determination of the SDA duration and magnitude, a curve fitting the postprandial $\dot{M}O_2$ values over time was generated in the program R version 3.0.2 (R Core Team 2013), employing a specific script developed by Chabot et al. (2016b) and applying the packages *quantreg* (Koenker 2015) and *Hmisc* (Harrell et al. 2015). The intercept of the curve with the individual SMR (yellow line, FIG. 17) marked the end of the SDA duration, while the magnitude was described by the integral below the curve (red shading, FIG. 17) (Chabot et al. 2016b). For details concerning settings within the R script, see publication III.

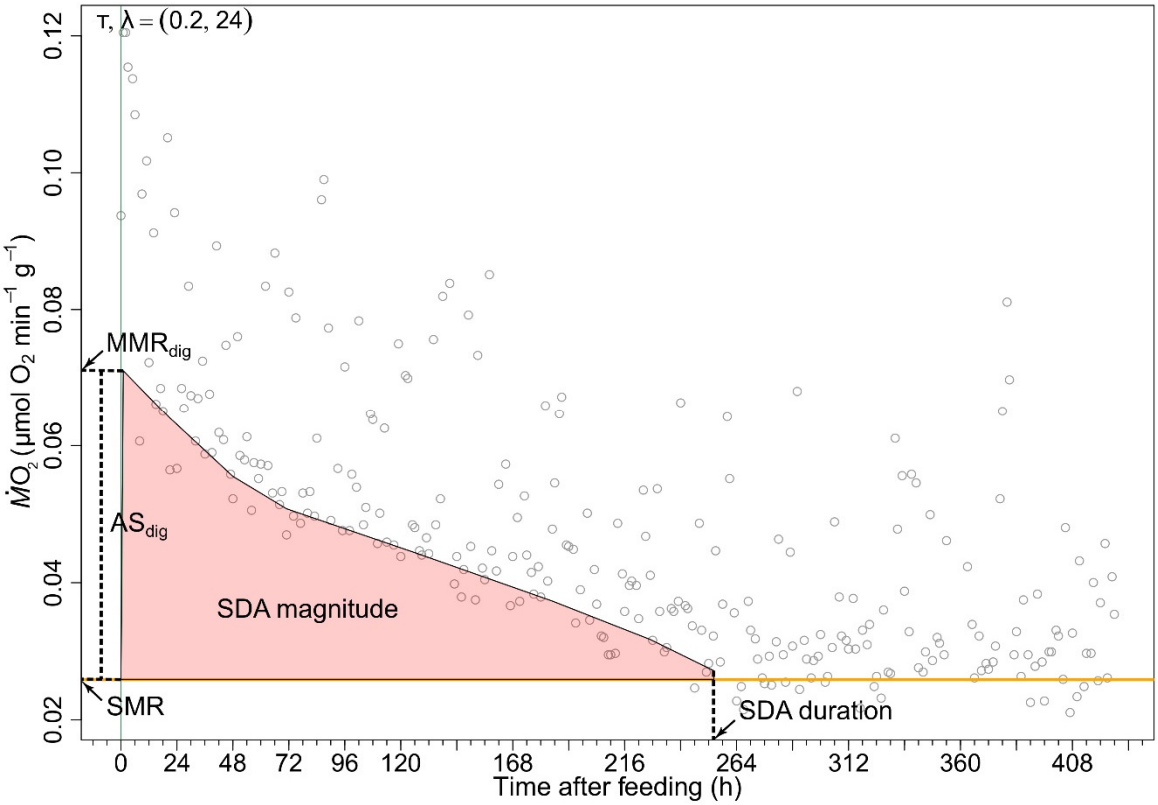

FIGURE 17. SDA profile including MMR_{dig}, SMR (20 % quantile, marked by yellow line), AS_{dig}, duration and magnitude (red area) (example: *B. saida*, 0°C).

2.6 Statistics

All statistical analyses were performed in the program R version 3.0.2 (R Core Team 2013) or in SigmaPlot 13 (Systat Software Inc, San Jose, California, USA). Shapiro-Wilk tests served to examine normal distribution. For the investigation of homoscedasticity, Bartlett (publication I) or Levene tests (publication II & III) were applied. Throughout the whole study, a significance level of $p < 0.05$ was accepted.

2.6.1 Incubation at different temperature/PCO_2 scenarios

Length and weight distributions at the beginning of the growth experiment, as well as swimming performance and respiratory capacities between temperature/PCO_2 treatments were compared by aid of a two-way ANOVA in R version 3.0.2 (R Core Team 2013). Further, temperature-effects within PCO_2 levels were analyzed for each parameter obtained during the growth experiment using one-way ANOVA. In case of significance, *post hoc* Tukey honest significance tests were performed subsequent to the ANOVA. Data sets characterized by unequal sample size and/or heterogeneous variances were investigated for temperature-effects using a max-t test (Herberich et al. 2010) by applying the R packages *multcomp* (Hothorn et al. 2008) and *sandwich* (Zeileis 2006).

Potential effects evoked by PCO_2 within the experimental temperatures were determined by Mann-Whitney U tests. In case of significant effects of PCO_2, two linear models (full interaction temperature and PCO_2, and reduced model PCO_2 plus interactive effect temperature:PCO_2, respectively) were fitted in order to characterize the effect of hypercapnia in a two-way approach. Similarly, Mann-Whitney U tests were applied to detect differences between species in all parameters (pooled across PCO_2 treatments) at 3 and 8°C. Furthermore, the overall data set of both species gained during the growth experiment was tested for impacts of temperature and PCO_2 with the aid of non-metric multidimensional scaling (NMDS) (for details and results, see publication I).

The mean burst count (BC) was expected to increase exponentially with velocity. Therefore, Sigmaplot 13 (Systat Software Inc, San Jose, California, USA) was used to find an exponential model to describe the BC throughout velocity steps at each temperature. The following model revealed the best fit (see publication II for significance levels):

$$\mathrm{BC\ (velocity)} = \mathrm{a\ exp}^{(\mathrm{b\ velocity})}$$

Significant differences in the burst performance between PCO_2-treatments were accepted in case of non-overlapping 95 % confidence intervals.

Data obtained from individuals heavily infested by parasites, from individuals that died during the incubation due to strict refusal of food (publication I), as well as data from fish with physical abnormalities or lethargic behavior (publication II) were omitted from statistical analysis. Further, *B. saida* that did not exhibit burst capacity were excluded during the statistical analysis of U_{crit} in order to maintain comparability to the variable U_{gait}.

2.6.2 Incubation at different temperatures and feeding conditions

A set of t-tests was performed in SigmaPlot 13 to reveal temperature effects on the SDA performance, as well as temperature and feeding effects on swimming and respiratory performance of *B. saida*. In case of heteroscedasticity, Mann-Whitney Rank Sum tests were performed (for details and results, see publication III).

Animals that refused to feed (SDA trial) or did not display a proper swimming behavior (swim trial) were excluded from statistical analysis. Further, individuals without burst capacity were not considered in statistics for the parameter U_{crit} in order to maintain comparability to the parameter U_{gait}.

3 Publications

The present thesis includes three first-authorship publications as well as two further contributions to publications (for the latter, see Appendix). Publications, authors and their contributions, as well as the current status of the articles are listed below.

PUBLICATION I

Authors: **Kristina Lore Kunz**, Stephan Frickenhaus, Silvia Hardenberg, Torild Johansen, Elettra Leo, Hans-Otto Pörtner, Matthias Schmidt, Heidrun Sigrid Windisch, Rainer Knust & Felix Christopher Mark

Title: New encounters in Arctic waters: a comparison of metabolism and performance of Polar cod (*Boreogadus saida*) and Atlantic cod (*Gadus morhua*) under ocean acidification and warming.

Status: published 2016 in *Polar Biology*, 39(6), 1137-1153,
doi: 10.1007/s00300-016-1932-z

Contributions: The idea for this study was developed by FCM, RK and HOP. The experimental set-up was designed by SH, FCM and myself. EL, MS and myself were responsible for animal maintenance and the documentation of water parameters. The experiments were performed by myself with the help of SH, EL, MS, HSW and FCM. SF and I analyzed the data. I interpreted the data with support from SF and FCM. The manuscript was written by myself and revised by all coauthors.

PUBLICATION II

Authors: **Kristina Lore Kunz**, Guy Claireaux, Hans-Otto Pörtner, Rainer Knust & Felix Christopher Mark

Title: Aerobic capacities and swimming performance of Polar cod (*Boreogadus saida*) under ocean acidification and warming conditions.

Status: published 2018 in *Journal of Experimental Biology*, 221(21), 1-11, doi: 10.1242/jeb.184473

Contributions: The idea for this study was developed by FCM, GC and myself. FCM, GC and myself designed the experimental protocols. The experiments were conducted by myself. I analyzed the data and interpreted them with the help of GC and FCM. The manuscript was written by myself and revised by GC, HOP, RK and FCM.

PUBLICATION III

Authors: **Kristina Lore Kunz**, Hans-Otto Pörtner, Rainer Knust & Felix Christopher Mark

Title: Influence of water temperature and feeding status on aerobic metabolic scope and swimming performance of Polar cod (*Boreogadus saida*).

Status: submitted to *Marine Biology*, May 2018

Contributions: The idea of this study was developed by myself. The experimental design was designed by myself with advice from FCM. I was responsible for animal maintenance and the implementation of the experiments. The data were analyzed by myself and discussed together with FCM. The manuscript was written by myself and revised by HOP, RK and FCM.

CONTRIBUTIONS TO ADDITIONAL PUBLICATIONS

PUBLICATION IV

Authors: Matthias Schmidt, Gabriele Gerlach, Elettra Leo, **Kristina Lore Kunz**, Steffen Swoboda, Hans-Otto Pörtner, Christian Bock & Daniela Storch

Title: Impact of ocean warming and acidification on the behaviour of two co-occurring gadid species, *Boreogadus saida* and *Gadus morhua*, from Svalbard

Status: published 2017 in *Marine Ecology-Progress Series*, 571, 183-191, doi: 10.3354/meps12130

PUBLICATION V

Authors: Elettra Leo, **Kristina Lore Kunz**, Matthias Schmidt, Daniela Storch, Hans-Otto Pörtner & Felix Christopher Mark

Title: Mitochondrial acclimation potential to ocean acidification and warming of Polar cod (*Boreogadus saida*) and Atlantic cod (*Gadus morhua*).

Status: published 2017 in *Frontiers in Zoology*, 14(1), 1–12, doi: 10.1186/s12983-017-0205-1

PUBLICATION I

New encounters in Arctic waters: a comparison of metabolism and performance of Polar cod (*Boreogadus saida*) and Atlantic cod (*Gadus morhua*) under ocean acidification and warming

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2016

Polar Biology, 39(6), 1137-1153

submitted: 16 October 2015
accepted: 14 March 2016
published: 24 March 2016
doi: 10.1007/s00300-016-1932-z

https://link.springer.com/article/10.1007/s00300-016-1932-z

Polar Biol (2016) 39:1137–1153
DOI 10.1007/s00300-016-1932-z

ORIGINAL PAPER

New encounters in Arctic waters: a comparison of metabolism and performance of polar cod (*Boreogadus saida*) and Atlantic cod (*Gadus morhua*) under ocean acidification and warming

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Received: 16 October 2015 / Revised: 9 March 2016 / Accepted: 14 March 2016 / Published online: 24 March 2016

Abstract Oceans are experiencing increasing acidification in parallel to a distinct warming trend in consequence of ongoing climate change. Rising seawater temperatures are mediating a northward shift in distribution of Atlantic cod (*Gadus morhua*), into the habitat of polar cod (*Boreogadus saida*), that is associated with retreating cold water masses. This study investigates the competitive strength of the co-occurring gadoids under ocean acidification and warming (OAW) scenarios. Therefore, we incubated specimens of both species in individual tanks for 4 months, under different control and projected temperatures (polar cod: 0, 3, 6, 8 °C, Atlantic cod: 3, 8, 12, 16 °C) and PCO_2 conditions (390 and 1170 µatm) and monitored growth, feed consumption and standard metabolic rate. Our results revealed distinct temperature effects on both species. While hypercapnia by itself had no effect, combined drivers caused nonsignificant trends. The feed conversion efficiency of normocapnic polar cod was highest at 0 °C, while optimum growth performance was attained at 6 °C; the long-term upper thermal tolerance limit was reached at 8 °C. OAW caused only slight impairments in growth performance. Under normocapnic conditions, Atlantic cod consumed progressively increasing amounts of feed than individuals under hypercapnia despite maintaining similar growth rates during warming. The low feed conversion

Electronic supplementary material The online version of this article (doi:10.1007/s00300-016-1932-z) contains supplementary material, which is available to authorized users.

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efficiency at 3 °C may relate to the lower thermal limit of Atlantic cod. In conclusion, Atlantic cod displayed increased performance in the warming Arctic such that the competitive strength of polar cod is expected to decrease under future OAW conditions.

Keywords Climate change · Gadoids · Hypercapnia · Thermal window · Growth · Feed consumption · RCP 8.5

Introduction

The oceans are currently experiencing rising water temperatures, and in parallel, the increase in atmospheric CO_2 concentrations causes ocean acidification. By the year 2100, global water surface temperatures are expected to rise by 2–3 °C paralleled by PCO_2 levels of up to 1370 µatm depending on future emission scenarios (RCP 8.5, Pörtner et al. 2014). Due to a higher solubility of CO_2 in cold waters and the freshening of surface waters, Arctic ecosystems more than other ecosystems are expected to be impaired by climate change. Organisms inhabiting high Arctic environments are in general well adapted to polar conditions; however, their narrow thermal windows imply high vulnerability under changing abiotic conditions.

The distribution of fish species is constrained by their species-specific temperature ranges (Coutant 1977; Pörtner 2002; Pörtner and Knust 2007; Pörtner and Peck 2010). Warming oceans cause a general trend of poleward movement of fish species (Brander et al. 2003; Parmesan 2006 and references therein) as reported for the Barents Sea (Drinkwater 2009), the North Sea (Perry et al. 2005), and the Bering Sea (Grebmeier et al. 2006). Invading species have a high potential to establish themselves in the previously colder areas as predicted by Parmesan (2006), provided that food availability, food quality, and potential spawning grounds support their settlement (Drinkwater 2005). Competition between invading and endemic taxa may alter well-established ecosystem structures and can force the local species to migrate into habitats with less favorable conditions (Renaud et al. 2012) thereby impairing fitness components such as growth and reproduction (Koehn and Shumway 1982). Ultimately, if under those conditions the long-term metabolic energy demand exceeds food supply (Brett 1979), growth and then population density will decline (Planque and Frédou 1999; Clark et al. 2003; Brander 2007).

Atlantic cod (*Gadus morhua*) (Perry et al. 2005; Drinkwater 2009) inhabits a broad geographical and thus thermal range (Scott 1982; Brander 1994, 1995), even within its different stocks (Malmberg and Blindheim 1994; Ottersen et al. 1998). Due to increasing water temperatures, Atlantic cod shift their habitat to the North (Kjesbu et al. 2014). The northernmost distribution boundary currently comprises the waters around Svalbard (Olsen et al. 2010). There, Atlantic cod co-occur with the resident polar cod (*Boreogadus saida*) (Renaud et al. 2012), which are associated with the retreating cold Arctic water masses (Gjøsæter 2009). Although direct competition for food is considered marginal (Renaud et al. 2012), potential interspecific interactions may occur via competition for space. Moreover, predation on polar cod is likely to increase with increasing abundance of Atlantic cod in Arctic waters (Renaud et al. 2012).

While Atlantic cod is one of the most exploited fish species in the North Atlantic (Drinkwater 2005; Brander 2010) and an important predator species (Björnsson et al. 2001), the most pronounced value of polar cod lies in its function as a prey species for several taxa including economically important fish species (Sekerak 1982; Bradstreet et al. 1986; Welch et al. 1993; Gjøsæter 2009). Any change in population size of one or both of these species may therefore have economic consequences together with unpredictable ecological interferences.

Individual growth and reproduction are the driving forces shaping population growth. Growth relies on excess energy which becomes available after baseline metabolic costs have been met. Later in life, beyond a specific body size, energy is required for gonadal development and reproduction (Pörtner et al. 2005).

Surplus energy for growth and reproduction, however, is only available within a species-specific temperature range. Within this range, the thermally stimulated appetite and food uptake support energy allocation to growth, linked to a species-specific optimum temperature for aerobic performance. At temperatures above the optimum for growth, food uptake is increasingly used to cover the exponentially increasing metabolic demands, resulting in declining growth rates (Brett 1979).

The interactions of growth, metabolic performance, and hypercapnia have mainly been studied in an aquaculture context at PCO_2 values far exceeding values projected for the open ocean (e.g. Fivelstad et al. 1998, 1999; Foss et al. 2003; Moran and Støttrup 2011). But even under realistic PCO_2 scenarios (700 µatm), reduced growth rates were found e.g., for Atlantic salmon parr (Fivelstad et al. 2007). According to the concept of oxygen- and capacity-limited thermal tolerance (OCLTT), narrower thermal windows and possibly lower performance optima have been proposed to occur under hypercapnia (Pörtner 2010). Furthermore, increased PCO_2 has been reported to reduce food consumption (Smart 1981; Crocker and Cech 1996; Foss et al. 2003) and at the same time may increase metabolic energy demand (Fivelstad et al. 2007).

Many studies report the effects of individual environmental stressors on the physiology of fish species,

especially in economically important species like Atlantic cod (e.g. Björnsson et al. 2001; Purchase and Brown 2001; Moran and Støttrup 2011). Knowledge of chronic and synergistic effects of ocean warming and acidification on the performance and fitness of fish species is still scarce, especially with respect to realistic future ocean conditions.

The aim of this study was to investigate the combined effects of projected ocean warming and acidification on growth performance, feed consumption, and standard metabolic rate in populations of two co-occurring gadoids, polar cod and Atlantic cod at their overlapping distribution ranges in the Arctic. The results are used to assess the competitive strength of both polar cod and Atlantic cod under projected water conditions. We discuss our findings in light of their ecology and the conditions faced by the species in their natural habitat.

Materials and methods

Sample collection

The polar cod used in this study were provided by the University of Tromsø, Norway. In January 2013, polar cod were caught from the R/V Helmer Hanssen with a bottom trawl in a depth of 120 m in Kongsfjorden (78° 97′N 12° 51′E) at the western coast of Svalbard. By aid of a fish lift (Holst and McDonald 2000), injuries during trawling were prevented. However, a mortality of approx. 50 % was recorded within the first week after capture. Size at capture was approx. 6–7 cm. The fish were kept in a flow-through seawater tank while being transported to the laboratories of Havbruksstasjonen i Tromsø AS (HiT) and kept until April 2013 at 3.3–3.8 °C under natural light conditions. They were fed three times a week with frozen copepods (*Calanus spec.*). In late April 2013, 150 individuals were transferred to the aquaria of the Alfred Wegener Institute (AWI) in Bremerhaven. In preparation for transportation, the fish were starved for a week.

The recirculating aquaria system at the AWI contained 10 m^3 of seawater originating from the North Sea near Helgoland (German Bight). Nitrification filters, protein skimmers and UV sterilizers as well as additional water changes were used to support high water quality and NO_3^- values below 50 mg L^{-1}. For preconditioning, the fish were kept at 5 °C for another 4 weeks. Starting one day after arrival, the polar cod were weaned onto a daily feeding pattern with high-protein feed pellets (Amber Neptun, 5 mm, Skretting AS, Norway). The light cycle was adjusted to 12 h of light and 12 h of darkness (12L:12D).

In August 2013, Atlantic cod were caught from the R/V Heincke at several locations in the vicinity of Svalbard: Rijpfjorden (80° 15.42′N 22° 12.89′E), Hinlopenstretet (79° 30.19′N 18° 57.51′E), and Forlandsundet (78° 54.60′N 11° 3.66′E) using a pelagic midwater trawl combined with a fish lift (Holst and McDonald 2000) at a depth of 0–40 m. Size at capture was approx. 5–7 cm. The fish were directly transported in a thermostatted recirculating tank system (4 m^3) to the AWI aquaria in Bremerhaven and kept for several months at 5 °C. Mortality during capture was low; however, cannibalism decreased the numbers of specimens substantially. They were fed twice a week with a mixture of frozen copepods, baby krill, and high-protein feed pellets.

Experimental design

The experimental design consisted of eight different stable temperature/PCO_2 treatments, each containing 12 single aquaria (approx. 24 L each). The chosen temperatures (polar cod: 0, 3, 6, 8 °C; Atlantic cod: 3, 8, 12, 16 °C) were based on the range of habitat temperatures and were maintained by the aid of thermostatted rooms. For each temperature, two PCO_2 levels were applied comprising the current conditions [390 µatm = control PCO_2; mean actual values: 430 µatm (polar cod), 480 µatm (Atlantic cod), Tables 1, 2] as well as a future value projected by the turn of the century [1170 µatm = high PCO_2; mean actual values: 1100 µatm (polar cod), 1120 µatm (Atlantic cod), Tables 1, 2] according to the Representative Concentration Pathway (RCP) 8.5 of the Intergovernmental Panel on Climate Change (IPCC) (Pörtner et al. 2014). For equal conditions across individual aquaria within the treatments, the respective PCO_2 conditions were pre-adjusted in a header tank containing approximately 200 L of seawater, supplying the single aquaria. The manipulation of CO_2 partial pressure was achieved by use of a mass flow controller (4 and 6 channel MFC system, HTK, Hamburg, Germany), in which virtually CO_2-free pressurized air was mixed with pure CO_2. This setup was identical for both species. It should be noted that this protocol differs from those traditionally applied in culture systems comprising a group of specimens in a common tank under the same environmental regime.

At the end of May 2013, 96 polar cod with a total length of 10.9–17.3 cm (mean length 14.2 cm ± 1.3 SD) and a weight of 6.7–31.9 g (mean weight 16.8 g ± 5.6 SD) were transferred to the experimental setup. Cooling and warming protocols were applied at a maximum rate of 2 °C per 24 h. To ensure proper technical replicates during allocation to experimental conditions, the distribution of individuals across temperatures, as well as PCO_2 regimes, was random. Each individual was placed into a single aquarium with an individual inflow of 500 mL min^{-1}.

The experiment with 96 Atlantic cod started in the end of April 2014. The size range was 14.2–24.8 cm (mean

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Table 1 Polar cod (*Boreogadus saida*)

Treatment (°C/μatm)	Salinity (psu)	TA (μmol/kgSW)	TCO_2 (μmol/kgSW)	pH_{tot}	PCO_2 (μatm)	HCO_3^- (μmol/kgSW)	CO_3^{2-} (μmol/kgSW)	ΩCa	ΩAr	Temp. (°C)
0/390	30.58 ± 0.98	2490.64 ± 81.02	2365.25 ± 76.27	8.08 ± 0.04	396.25 ± 37.70	2237.39 ± 72.19	103.37 ± 10.47	2.52 ± 0.25	1.57 ± 0.16	0.90 ± 0.37
0/1170	30.70 ± 1.08	2459.25 ± 69.68	2466.79 ± 76.22	7.66 ± 0.05	1091.64 ± 131.67	2357.46 ± 71.32	41.30 ± 3.66	1.01 ± 0.09	0.63 ± 0.06	0.61 ± 0.45
3/390	30.70 ± 1.01	2480.35 ± 45.21	2352.84 ± 39.80	8.05 ± 0.04	431.10 ± 40.41	2223.17 ± 37.20	105.22 ± 8.68	2.56 ± 0.21	1.60 ± 0.13	3.15 ± 0.18
3/1170	30.74 ± 0.98	2447.33 ± 48.03	2447.22 ± 52.89	7.65 ± 0.05	1135.99 ± 147.10	2338.40 ± 49.31	44.35 ± 4.17	1.08 ± 0.10	0.67 ± 0.06	3.06 ± 0.11
6/390	31.47 ± 0.53	2371.73 ± 159.40	2237.56 ± 148.24	8.03 ± 0.02	437.09 ± 21.60	2107.92 ± 137.19	107.16 ± 11.27	2.60 ± 0.28	1.63 ± 0.17	5.63 ± 0.19
6/1170	31.49 ± 0.52	2353.22 ± 165.66	2342.31 ± 164.82	7.64 ± 0.03	1123.50 ± 86.23	2237.51 ± 157.89	46.82 ± 4.79	1.14 ± 0.12	0.71 ± 0.07	5.57 ± 0.21
8/390	32.78 ± 0.82	2528.77 ± 135.07	2357.44 ± 127.60	8.05 ± 0.02	440.90 ± 29.10	2202.98 ± 118.79	133.74 ± 10.36	3.22 ± 0.25	2.03 ± 0.16	8.16 ± 0.13
8/1170	32.71 ± 0.80	2531.76 ± 115.67	2482.53 ± 114.04	7.71 ± 0.05	1052.88 ± 103.87	2364.26 ± 113.98	68.87 ± 17.75	1.66 ± 0.43	1.05 ± 0.27	8.11 ± 0.13

Water chemistry for all treatments as mean ± SD throughout the total experimental period of 4 months

Table 2 Atlantic cod (*Gadus morhua*)

Treatment (°C/μatm)	Salinity (psu)	TA (μmol/kgSW)	TCO_2 (μmol/kgSW)	pH_{tot}	PCO_2 (μatm)	HCO_3 (μmol/kgSW)	CO_3^{2-} (μmol/kgSW)	ΩCa	ΩAr	Temp. (°C)
3/390	31.62 ± 0.41	2216.73 ± 113.32	2111.36 ± 106.46	7.99 ± 0.04	449.08 ± 39.04	2000.34 ± 99.99	86.36 ± 9.20	2.09 ± 0.22	1.31 ± 0.14	3.00 ± 0.20
3/1170	31.60 ± 0.37	2194.07 ± 112.10	2196.88 ± 101.83	7.61 ± 0.07	1127.75 ± 164.89	2096.49 ± 99.83	38.83 ± 7.14	0.94 ± 0.17	0.59 ± 0.11	2.98 ± 0.20
8/390	33.01 ± 0.35	2267.67 ± 64.73	2123.50 ± 56.92	8.01 ± 0.04	434.51 ± 49.90	1992.24 ± 52.62	110.54 ± 11.08	2.66 ± 0.26	1.68 ± 0.17	8.16 ± 0.11
8/1170	32.70 ± 1.35	2255.23 ± 62.17	2210.37 ± 50.37	7.72 ± 0.08	915.08 ± 127.39	2106.92 ± 46.88	59.73 ± 11.09	1.40 ± 0.22	0.88 ± 0.14	8.07 ± 0.17
12/390	32.14 ± 0.50	2431.18 ± 88.61	2261.45 ± 80.24	8.00 ± 0.08	490.29 ± 112.75	2108.17 ± 78.43	132.63 ± 22.10	3.21 ± 0.54	2.04 ± 0.34	12.03 ± 0.35
12/1170	32.15 ± 0.48	2434.19 ± 39.81	2391.13 ± 37.28	7.66 ± 0.07	1163.71 ± 199.68	2277.80 ± 36.50	64.14 ± 9.36	1.55 ± 0.23	0.98 ± 0.14	11.85 ± 0.37
16/390	32.61 ± 0.37	1930.37 ± 170.37	1809.62 ± 164.23	7.87 ± 0.06	547.87 ± 92.69	1697.86 ± 155.30	91.40 ± 13.81	2.21 ± 0.33	1.41 ± 0.21	15.81 ± 0.25
16/1170	32.61 ± 0.38	1921.64 ± 175.50	1897.43 ± 186.68	7.54 ± 0.10	1271.67 ± 365.09	1803.91 ± 178.67	46.16 ± 9.76	1.12 ± 0.23	0.71 ± 0.15	15.81 ± 0.27

Water chemistry for all treatments as mean ± SD throughout the total experimental period of 4 months

total length 18.5 cm ± 2.2 SD) and weight was 15.3–103.8 g (mean weight 43.4 g ± 17.3 SD). Temperature adjustments to reach final experimental conditions did not exceed 1 °C per 24 h. The protocol of distributing individuals in the single aquaria was identical between both incubations.

Spatial constraints within the culture rooms forced us to isolate individual treatments (polar cod: 0 and 3 °C; Atlantic cod: 12 °C) from the total recirculating water body. Nonetheless, each system contained approx. 1200 L of recirculating seawater preconditioned with respect to temperature and PCO_2. Apart from this, setups were identical to those described above. NH_4^+ tests were conducted twice a week using photometric test kits (Macherey–Nagel, Düren, Germany). The critical threshold for water changes was set to 0.4 mg L^{-1} for NH_4^+. During the incubation with Atlantic cod, water quality was maintained by biological filter systems in combination with protein skimmers (Sander, Germany) and daily water exchanges (600 L).

Water chemistry

The stability of PCO_2 conditions was verified by monitoring pH, temperature, and salinity once to twice a week in triplicate in every treatment. For pH measurements, a pH meter (pH 3310, WTW, Weilheim, Germany) was calibrated with thermally equilibrated NBS-buffers (2-point calibration). The pH values were cross-calibrated to total pH scale using Tris-buffered pH reference material (Batch 4, Marine Physical Laboratory, University of California, San Diego, CA, USA). Temperature and salinity were measured using a WTW LF 197 multimeter (WTW, Weilheim, Germany).

In combination with the total dissolved inorganic carbon (verified in triplicates) determined by a Seal QuAAtro SFA Analyzer (800 TM, Seal Analytical, Mequon, USA), the pH_{tot} values were used to subsequently calculate the seawater carbonate chemistry in the program CO2SYS (Lewis and Wallace 1998), applying the dissociation constants of Mehrbach et al. (1973) refitted by Dickson and Millero (1987) and the $KHSO_4$ constant after Dickson (1990). A summary of water chemistry data is given in Tables 1 and 2.

Growth experiment

In total, the growth experiment lasted 130 days (polar cod) and 133 days (Atlantic cod) under constant conditions. For the determination of individual growth rates, each specimen was measured (total length; to the nearest mm below) and weighed (wet weight; to the nearest 0.1 g) on the third day after feeding, three times during the experiment: (1) prior to the transfer into the experimental setup, (2) after the incubation half-time (polar cod, day 60–68; Atlantic cod, day 44–47), and (3) at the end of the incubation period (polar cod, day 109–130; Atlantic cod, day 108–133). For the precise determination of length and weight, the fish were slightly sedated (0.06 g L^{-1} MS-222) prior to measuring. A light cycle of 12:12 h was maintained. During day time, the light was dimmed except for the feeding and feed-removing events, as well as during water condition measurements.

Each fish was fed ad libitum once every 4 days using a predetermined amount of high-protein feed pellets (Amber Neptun, 5 mm, Skretting AS, Norway). For a better feed acceptance of polar cod, the pellets were soaked with a fixed amount of sea water 24 h before feeding. Remaining feed items were removed after several hours, dried for 24 h at 80 °C, and weighed for the quantification of individual feed intakes. The relative nutrient composition of the pellets was 54.7 % protein, 19.1 % fat, 8.0 % carbohydrates, and 8.5 % moisture. Every single aquarium was cleaned daily from feces and other particles. In the case of feeding days, cleaning happened well before the feeding events.

Respiration measurements

At the end of the long-term incubation, individual oxygen consumption rates [$\dot{M}O_2$; unit: µmol (min*g)$^{-1}$] were determined (polar cod, $n = 4$–6; Atlantic cod, $n = 5$–12 per treatment) using automated intermittent-flow respirometry. Two systems comprising six acryl respiration chambers each (polar cod, 1.8 and 2.2 L; Atlantic cod, 3.9 L) were installed. The respiration chambers were submerged in water of the respective temperature/PCO_2 treatments. A circulating water flow was constantly maintained within the respiration chambers by aid of an aquarium pump (8.2 L min^{-1}). A flush pump (5.0 L min^{-1}) was used to fully replenish the O_2 concentration in the chambers after a measurement period of 15 min (polar cod; Atlantic cod, 3 and 8 °C) and 10 min (Atlantic cod, 12 and 16 °C). $\dot{M}O_2$ was measured using optical oxygen probes and recorded with a 10-channel oxygen meter (PreSens Precision Sensing GmbH, Hamburg, Germany; system 1), as well as a four-channel FireStingO2 (Pyro Science GmbH, Aachen, Germany) and two Fibox 3 (PreSens Precision Sensing GmbH, Hamburg, Germany) (system 2). For calibration, the oxygen probes were flushed with nitrogen at room temperature (0 %-calibration), and the 100 %-calibration was performed in fully aerated seawater at the respective experimental temperature prior to each round of measurements. Blank measurements detected bacterial background respiration following $\dot{M}O_2$ measurements at each temperature (polar

cod) or at each temperature/PCO_2 combination (Atlantic cod) during several loops. After subtraction of bacterial respiration (solely detected at 8 °C for polar cod and 16 °C for Atlantic cod), the average of the five lowest, consecutive $\dot{M}O_2$ values per individual was used as an estimate of standard metabolic rate (SMR).

The fish were placed in the respiration chambers on the third day after feeding and remained in the chambers for approx. 48 h to allow for full recovery from handling stress. During the measurement period, non-transparent plastic sheets covered the tanks containing the respiration chambers to reduce potential disturbance. In order to minimize the time of air exposure, weighing was conducted before the fish were placed in the chambers, while length measurements happened after the determination of $\dot{M}O_2$.

Statistical analysis

The specific growth rate (SGR) per day was calculated in percent of the initial weight according to Jobling (1988):

$$\mathrm{SGR} = 100 * (\ln W_{\mathrm{end}} - \ln W_{\mathrm{start}}) * (t_{\mathrm{end}} - t_{\mathrm{start}})^{-1},$$

with W_{start} and W_{end} being the individual weight in gram at the day t_{start} and t_{end}, respectively.

The individual feed intake (F) per meal was calculated according to:

$$F = \mathrm{hl} * F_{\mathrm{in}} - F_{\mathrm{out}}$$

The constant (hl) supports the compensation of different humidity levels of feed pellets before and after drying. The determination of exact humidity levels in feed pellets prior to both incubations revealed slightly divergent correction factors (hl = 0.8820 and 1.0227 during the incubation with polar cod and Atlantic cod, respectively). F_{in} is the amount of feed given to the respective individual per meal, while F_{out} represents the amount of remaining feed items in gram. Subsequently, the feed intake per body weight (weight at experimental midterm) of each individual throughout the whole experimental period was determined. Furthermore, individual stomachs and their content were weighed the third day after the last feeding event in order to determine the degree of stomach filling (SF) in percent of the stomach weight.

Feed conversion ratio (FCR) of each fish was calculated as the individual weight gain divided by the individual feed intake throughout the experiment.The condition factor (CF) was calculated according to Fulton (1911):

$$\mathrm{CF} = 100 * W * L_{\mathrm{t}}^{-3},$$

where W is the wet weight in gram and L_{t} represents the total length in centimeter.

The hepatosomatic index (HSI) was calculated as:

$$\mathrm{HSI} = 100 * W_{\mathrm{L}} * W^{-1},$$

with W_{L} representing the liver weight in gram.

The gonadosomatic index (GSI) was determined according to:

$$\mathrm{GSI} = 100 * W_{\mathrm{G}} * W^{-1},$$

with W_{G} representing the gonad weight in gram.

Prior to statistical analysis, individuals that were heavily infested by parasites or that died during the incubation due to total refusal of feed were excluded from the data set.

All of the following tests were accomplished using the program R version 3.0.2 (R Core Team 2013). $p < 0.05$ is considered as significant. Comparisons of the initial mean weight and length of each fish species between experimental treatments were conducted using a two-way ANOVA. Normal distribution and homoscedasticity were assessed by Shapiro–Wilk tests and Bartlett tests, respectively.

A one-way ANOVA was performed to test for temperature-dependent effects within both PCO_2 levels for each species. In case of significant effects, a subsequent Tukey honest significance test for comparisons of the mean was applied. When the data set was characterized by unequal sample sizes and/or heterogeneous variances (F, SF, FCE, CF, GSI and SMR), a max-t test (Herberich et al. 2010) was conducted to assess temperature effects within the PCO_2 levels. The procedure was performed using a combination of the R packages MULTCOMP (Hothorn et al. 2008) and SANDWICH (Zeileis 2006).

PCO_2-dependent effects on SGR, F, SF, FCE, CF, HSI, GSI, and SMR within each temperature were investigated by aid of Mann–Whitney U tests. In case of significant effects of PCO_2, two linear models (full interaction temperature and PCO_2, and reduced model PCO_2 plus interactive effect temperature: PCO_2, respectively) were fitted in order to characterize the effect of hypercapnia in a two-way approach.

When no effects of PCO_2 were detected within each species, a second Mann–Whitney U test was done for species comparisons in pooled data across PCO_2 treatments for SGR, F, FCE, and SMR at 3 and 8 °C, respectively.

The overall data set from both species was tested for the impact of temperature and PCO_2 with the aid of non-metric multidimensional scaling (NMDS). The parameter GSI was excluded in this analysis, because the sex-specific response was shown to outperform every effect of temperature and PCO_2 in preliminary tests. Furthermore, HSI was excluded, because HSI caused the discard of the 16 °C treatments of Atlantic cod in the NMDS, which entailed a reduction in the power of the NMDS. The NMDS showed stress 0.11 for polar cod and 0.08 for Atlantic cod.

Results

For both species, initial mean fish weight and length did not show any differences between the treatments.

Mortality

Polar cod mortality occurred solely at the highest temperature (8 °C, $n = 3$, ≙13.0 %) at a rather late stage of the incubation (Fig. 1). Dead Atlantic cod ($n = 12$, ≙12.5 %) were recorded at all temperatures and PCO_2 values with a peak of four individuals (33.3 %) at 12 °C/390 µatm. Highest mortality of Atlantic cod ($n = 8$, ≙8.3 %) occurred within the first half of the incubation period (Fig. 1).

Growth

Growth performance of polar cod did not show any statistically significant temperature effect (Fig. 2). However, under control PCO_2 conditions a trend for higher growth at 6 °C was recognizable ($p = 0.07$). No such temperature trend was found under hypercapnia ($p = 0.6$). The growth rate of Atlantic cod was strongly correlated with temperature (control PCO_2: $p < 0.0001$; high PCO_2: $p < 0.0001$) with a high slope at low and a decreasing slope at higher temperatures (Fig. 2). Therefore, no thermal optimum for growth could be recorded between 3 and 16 °C. A comparison of both species revealed no significant difference in growth rates at 3 °C ($W = 361$, $p = 0.07$). At 8 °C, the growth performance of Atlantic cod was higher than that of polar cod ($W = 46$, $p < 0.0001$).

Feed consumption

For both species, the data set of daily feed consumption per gram body weight was influenced by temperature (Fig. 3). The feed intake of polar cod was lowest at 0 °C (mean = 3.5 mg (g BW*day)$^{-1}$) compared to the other experimental temperatures, a difference which was more pronounced under hypercapnia (control PCO_2: 0/3 °C $p = 0.06$, 0/6 °C $p = 0.04$, 0/8 °C $p = 0.1$; high PCO_2: 0/3 °C $p = 0.02$, 0/6 °C $p = 0.02$, 0/8 °C $p = 0.001$). At 3, 6 and 8 °C, the feed consumption plateaued and reached an average of 4.8 mg (g BW*day)$^{-1}$. The degree of stomach filling the third day after feeding in polar cod decreased nonsignificantly with increasing temperature. The daily feed consumption per g body weight of Atlantic cod rose with temperature, covering a range from 5.1 (3 °C) to 11.2 mg (g BW*day)$^{-1}$ (16 °C). Under hypercapnia, the slope was progressively less pronounced at high temperatures compared to normocapnic conditions. Atlantic cod consumed distinctly more feed at 8 °C ($W = 46$, $p < 0.0001$) than polar cod. The third day after feeding, stomach contents of Atlantic cod were low except for nonsignificantly higher amounts at 3 °C.

Feed conversion efficiency

The temperature effect on growth and/or feed intake was also translated into the feed conversion ratio for both

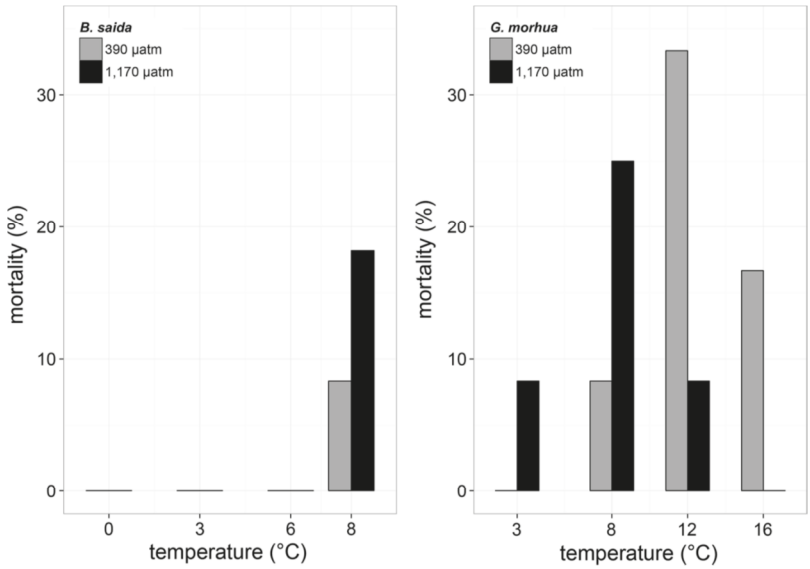

Fig. 1 Total mortality (%) during long-term incubation including specimens that refused feed consumption from the beginning of the experimental period (excluded in the analysis of further parameters)

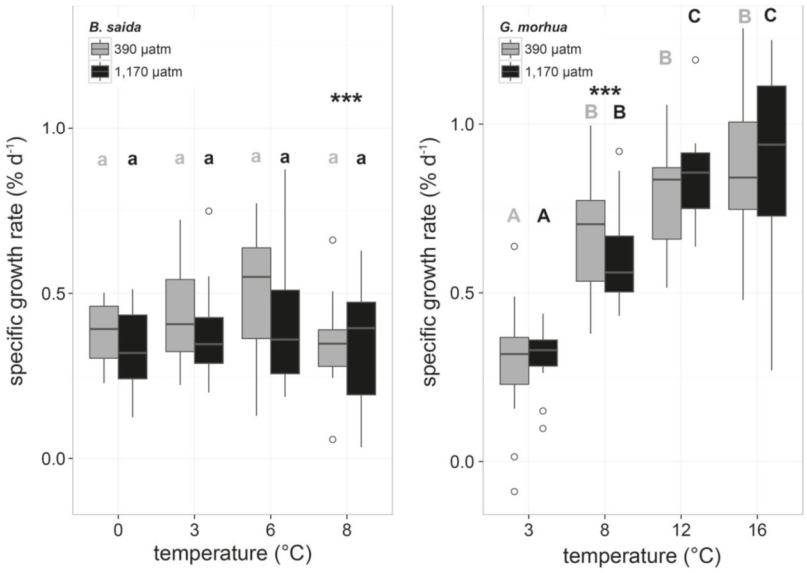

Fig. 2 Specific growth rate (wt% d^{-1}). *Letters* comparison of temperature treatments at different PCO_2 levels (390 µatm: *gray letters*; 1170 µatm: *black letters*) in polar cod (*Boreogadus saida*) (*lowercase*) and Atlantic cod (*Gadus morhua*) (*uppercase*). *Asterisks* significant difference between species at 8 °C; ***: $p < 0.001$

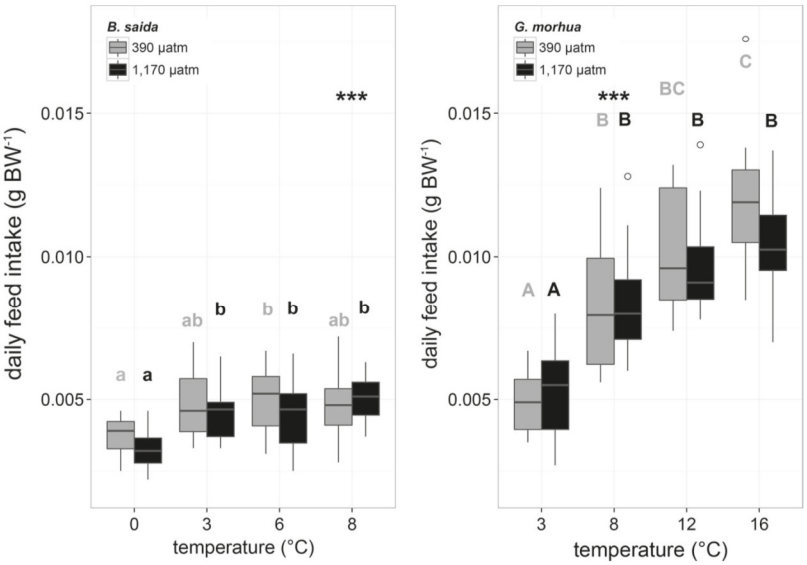

Fig. 3 Daily feed consumption (g BW^{-1}). *Letters* comparison of temperature treatments at different PCO_2 levels (390 µatm: *gray letters*; 1170 µatm: *black letters*) in polar cod (*lowercase*) and Atlantic cod (*uppercase*). *Asterisks* significant difference between species at 8 °C; ***: $p < 0.001$

species (Fig. 4). A nonsignificant trend for feed conversion efficiency of polar cod to be highest was recognizable at 0 °C (mean = 1.18). Feed conversion fell to its lowest value at 8 °C (mean = 0.71; control PCO_2: 0/8 °C $p = 0.001$, 3/8 °C $p = 0.06$, 6/8 °C $p = 0.02$; high PCO_2: 0/8 °C $p = 0.02$, 3/8 °C $p = 0.4$, 6/8 °C $p = 0.4$). Atlantic cod revealed a stable feed conversion ratio at 8, 12 and 16 °C with an average value of 0.97. An exceptionally low feed conversion efficiency was found at 3 °C, which was most distinct under hypercapnia (mean = 0.65; control PCO_2: 3/8 °C $p = 0.04$, 3/12 °C $p = 0.1$, 3/16 °C $p = 0.1$; high PCO_2: 3/8 °C $p = 0.005$, 3/12 °C $p = 0.001$, 3/16 °C $p = 0.001$). Therefore, polar cod showed a higher feed conversion ratio at 3 °C than Atlantic cod ($W = 439$, $p = 0.0005$), while the opposite was the case at 8 °C ($W = 108$, $p = 0.001$).

Condition factor and hepatosomatic index

Polar cod revealed an inverse relationship between CF and temperature. In contrast, the CF of Atlantic cod increased with temperature (Fig. 5).

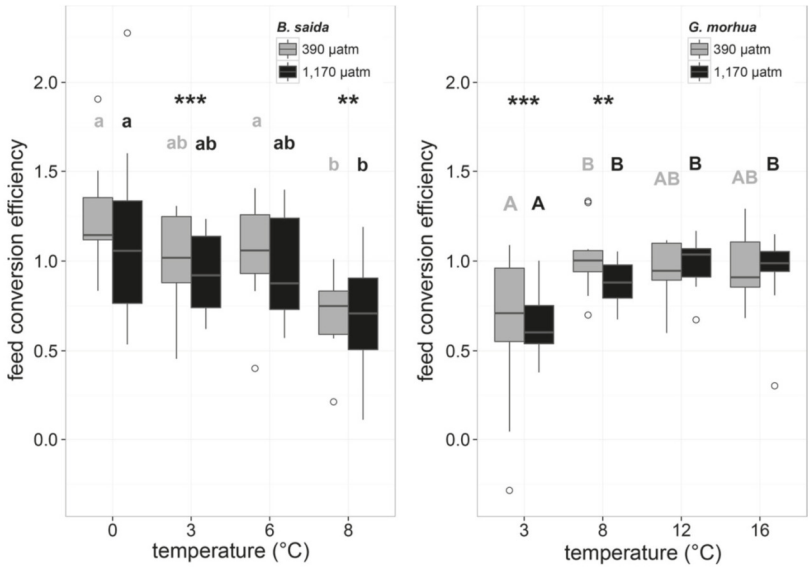

Fig. 4 Feed conversion efficiency. *Letters* comparison of temperature treatments at different PCO_2 levels (390 µatm: *gray letters*; 1170 µatm: *black letters*) in polar cod (*lowercase*) and Atlantic cod (*uppercase*). *Asterisks* significant difference between species at 3 and 8 °C, respectively; **: $p < 0.01$, ***: $p < 0.001$

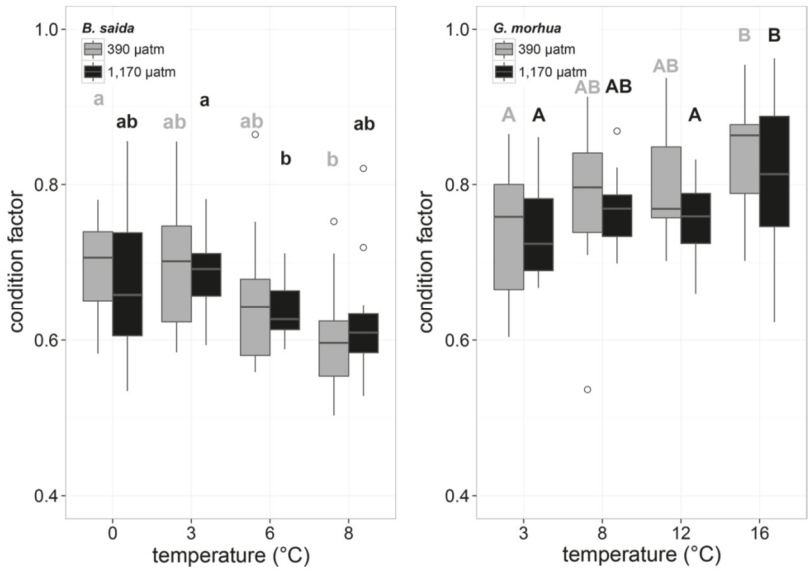

Fig. 5 Condition factor according to Fulton. *Letters* comparison of temperature treatments at different PCO_2 levels (390 µatm: *gray letters*; 1170 µatm: *black letters*) in polar cod (*lowercase*) and Atlantic cod (*uppercase*)

The trend of an increasing HSI at higher temperatures could statistically not be supported for both polar cod and Atlantic cod under normocapnia. Under high PCO_2, the increase in HSI with temperature was more pronounced in Atlantic cod ($p < 0.0001$) (Fig. 6), resulting in a significantly higher HSI under high PCO_2 than at control PCO_2 at 16 °C ($W = 0$, $p = 0.008$). The full linear model showed no significant temperature dependency for both species (polar cod $p = 0.14$, Atlantic cod $p = 0.1$). The reduced linear model supported the findings on PCO_2 impact, taking into consideration the whole range of investigated temperatures and testing the interaction term (polar cod: $p = 0.8$; Atlantic cod: $p = 0.0004$; Online Resource 1). Atlantic cod shows a significant positive slope (0.34151) in its temperature-dependent trend of HSI under elevated PCO_2 matching the trend seen in Fig. 6.

Gonadosomatic index

Sex determination revealed a majority of immature males (85.2 %) among polar cod and a slightly larger fraction of immature females (67.2 %) among Atlantic cod. The GSI

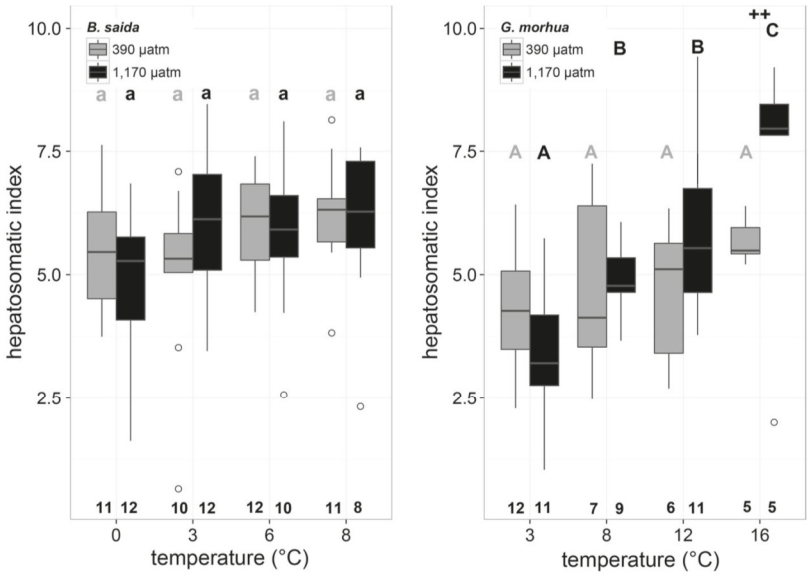

Fig. 6 Hepatosomatic index. Polar cod (n = 8–12); Atlantic cod (n = 5–12). *Letters* comparison of temperature treatments at different PCO_2 levels (390 µatm: *gray letters*; 1170 µatm: *black letters*) in polar cod (*lowercase*) and Atlantic cod (*uppercase*). *Plus sign* significant difference between PCO_2 treatments at the same temperatures within the species; ++: $p < 0.01$. Numbers below = n

of male polar cod and its variability tended to decrease with increasing temperature. At 0 °C, the average value was 7.31 %. The GSI of female Atlantic cod did not show any variation with temperature and appeared to be relatively low (mean = 0.34 %; Fig. 7; GSI data for complementary sexes of both species are shown in the figure in Online Resource 2).

Standard metabolic rate

The SMR of both species was strongly affected by temperature (Fig. 8). Polar cod acclimated for 4 months to the respective temperature showed a similar SMR at 0, 3 and 6 °C (mean = 0.053 µmol (min*g)$^{-1}$), but at 8 °C, SMR was strongly enhanced (mean = 0.080 µmol (min*g)$^{-1}$). This effect was most distinct under normocapnic conditions (control PCO_2: 0/8 °C $p = 0.01$, 3/8 °C $p = 0.002$, 6/8 °C $p = 0.02$; high PCO_2: 0/8 °C $p = 0.02$, 3/8 °C $p = 0.03$, 6/8 °C $p = 0.05$). The SMR of Atlantic cod increased with temperature from 0.030 (3 °C) to 0.068 µmol (min*g)$^{-1}$ (16 °C). At both 3 and 8 °C, SMR was distinctly higher in polar cod than in Atlantic cod (3 °C: $W = 247$, $p < 0.0001$; 8 °C: $W = 189$, $p < 0.0001$).

Effect of temperature and PCO_2 on the global data set

A NMDS analysis of the global data set revealed a significant impact of temperature in both species (polar cod: $p = 0.001$, Atlantic cod: $p = 0.001$; Fig. 9). A significant effect of PCO_2 was only detected in the total data set of polar cod ($p = 0.03$).

Discussion

Few studies are available that investigated growth performance of fish under predicted moderate PCO_2 conditions. In general, vulnerability of marine teleosts under PCO_2 conditions projected for the year 2100 is considered to be low due to excess capacities for acid–base regulation in gill cells (Melzner et al. 2009; Michael et al. 2016). Besides that, the life stages investigated in the present study are less sensitive to changing abiotic conditions than eggs, larvae or spawning life stages (Pörtner and Farrell 2008). Accordingly, neither mortality nor growth performance of polar cod and Atlantic cod were significantly influenced by chronic exposure to hypercapnia in this study. However, nonsignificant trends caused by hypercapnia were found in most of the parameters investigated. As a note of caution, the impact of PCO_2 might be reduced by low growth performance due to low-frequency feeding events. In polar cod, the effect of PCO_2 was visible in a nonsignificant trend for depressed growth performance under high PCO_2 conditions, which was most pronounced at the optimum temperature for growth determined under control conditions (6 °C). Costly compensatory processes in ion and acid–base regulation may cause the decrease in scope for growth. Growth impairment attributed to decreased food consumption under hypercapnic conditions (Smart 1981) may be the result of an uncompensated respiratory acidosis

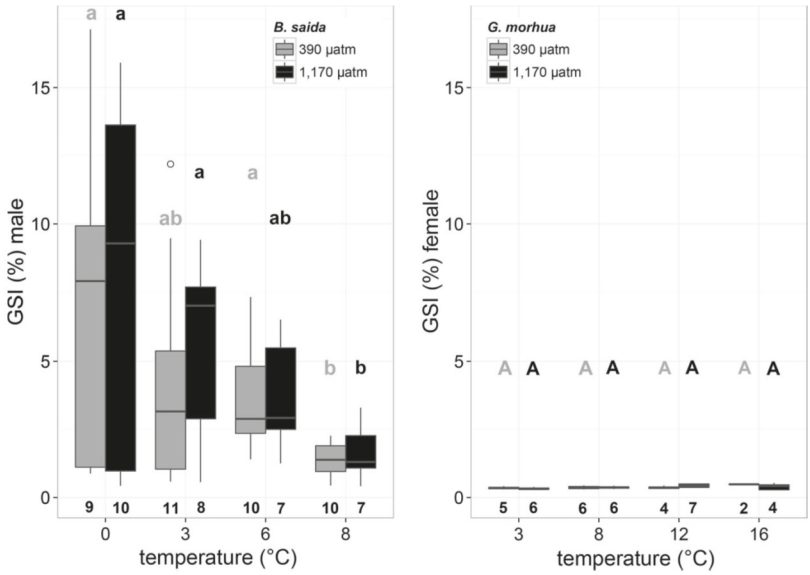

Fig. 7 Gonadosomatic index (%). Polar cod, male ($n = 7$–11); Atlantic cod, female ($n = 2$–7). *Letters* comparison of temperature treatments at different PCO_2 levels (390 µatm: *gray letters*; 1170 µatm: *black letters*) in polar cod (*lowercase*) and Atlantic cod (*uppercase*). Numbers below = *n*

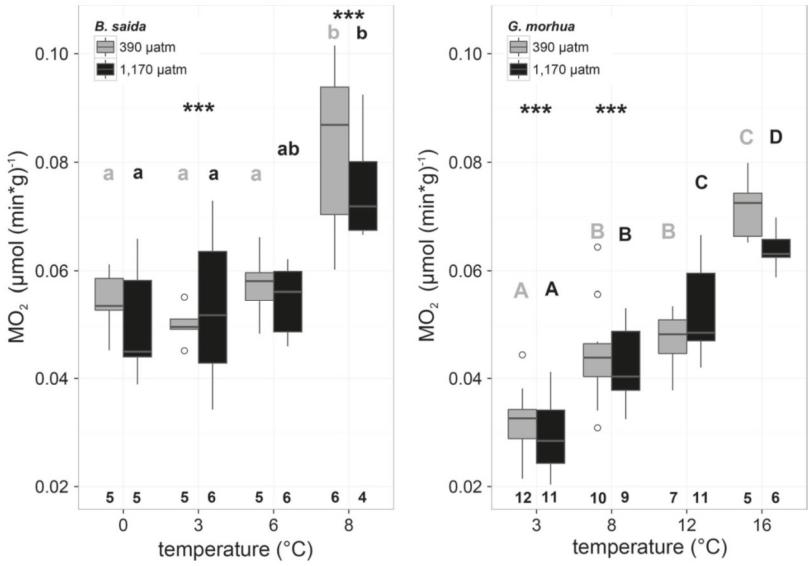

Fig. 8 Standard metabolic rate (µmol (min*g)$^{-1}$). Polar cod ($n = 4$–6); Atlantic cod ($n = 5$–12). *Letters* comparison of temperature treatments at different PCO_2 levels (390 µatm: *gray letters*; 1170 µatm: *black letters*) in polar cod (*lowercase*) and Atlantic cod (*uppercase*). *Asterisks* significant difference between species at 3 and 8 °C, respectively; ***: $p < 0.001$. Numbers below = *n*

(Crocker and Cech 1996). While no such trend was detected in polar cod, a (nonsignificant) trend for depressed feed intake under hypercapnia was recorded for Atlantic cod and became more prominent in the warmth. Nevertheless, a decline in growth performance or feed conversion efficiency was not detected in Atlantic cod of our study. Furthermore, hypercapnia caused a nonsignificant decrease in metabolic rate as well as a significantly enhanced HSI compared to the control treatment at 16 °C. Liver enlargement might be a compensatory response to potentially impaired lipase activity (Yada et al. 2002) under high PCO_2 conditions. An alternative hypothesis attributes the enhanced HSI to an energy surplus, potentially evoked by the amount of energy conserved due to suppressed foraging activity in response to elevated CO_2 levels. This hypothesis indicates that the decline in feed uptake of Atlantic cod is less than the decline in energy demand due to foraging activity such that Atlantic cod is thriving slightly better under hypercapnic conditions close to its thermal optimum for growth. Although this

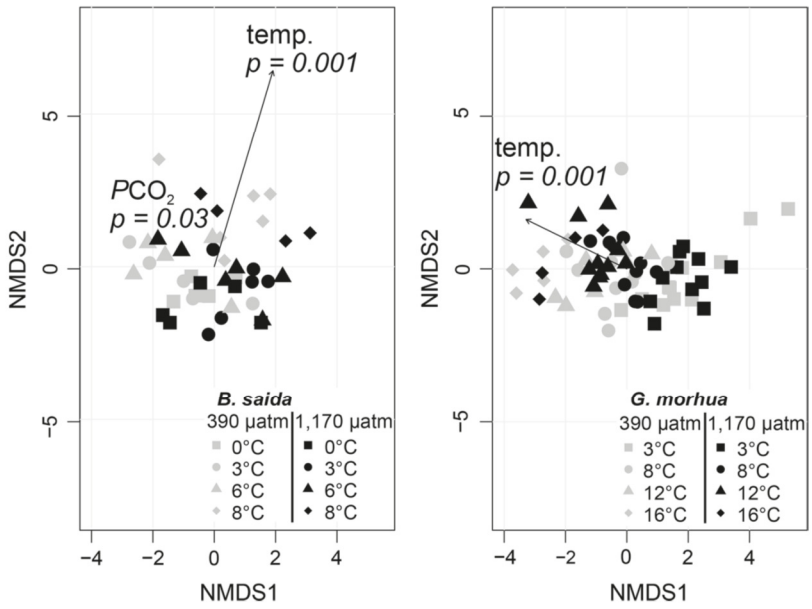

Fig. 9 Result of non-metric multidimensional scaling. Investigation of effects of temperature and PCO_2 on the global data set (excluding GSI and HSI)

explanation remains speculative, if true it may reflect the wide range of abiotic habitat conditions experienced within the lifetime of Atlantic cod (Neuenfeldt et al. 2009). For comparison, polar cod faces less extreme variations in habitat PO_2 and PCO_2. Hence, the onset of growth impairment of polar cod under hypercapnia indicates a higher sensitivity of this species to projected future PCO_2 values, compared to the more tolerant Arctic population of Atlantic cod. This conclusion is supported by the outcome of the NMDS analysis of the overall data set.

We kept individuals isolated from each other due to cannibalism recorded in the common tanks prior to the start of the experiment. Thereby, their feeding efforts (including foraging and competition) were minimized to below levels typical for their natural environment and aquaculture conditions. This may have improved their growth performance overall and at higher temperatures. The temperature profiles detected here can thus not easily be transferred to the natural environment but provide relative information on the competitive strength of the two species with respect to growth.

For polar cod, mortality occurred exclusively at the highest investigated temperature (8 °C) at a late stage of the experiment. This indicates that moderate constraints on the animal's well-being had long-term effects, classifying 8 °C as the species' long-term upper thermal tolerance limit. Protective mechanisms such as heat-shock proteins, use of anaerobic metabolism, and antioxidative defense are likely to only support time-limited periods of passive tolerance as outlined by the OCLTT concept (Pörtner 2012). Elevated baseline energy turnover leads to reduced growth and, finally, via whole organism and cellular functional constraints and stress, reduced survival rates in polar cod at 8 °C. This contrasts earlier findings by Christiansen et al. (1996) who reported that polar cod survives temperatures up to 14 °C under laboratory conditions. Unfortunately, the authors neither specified the initial acclimation temperature nor the exact duration in the warming protocol. Higher lethal temperatures during shorter-term warming protocols, however, seem to be a general phenomenon and were also found by Peck et al. (2009) in polar species.

Polar cod is known to be a slow growing species (Hop et al. 1997a; Gjøsæter 2009), explaining the limited maximum body length of approx. 30 cm despite a maximum lifespan of 7 years (Bradstreet et al. 1986; Hop et al. 1997b). Furthermore, growth rates decrease with increasing body weight and age (e.g. Jobling 1983, 1988; Björnsson and Steinarsson 2002; Björnsson et al. 2007). Size-at-age data (Falk-Petersen et al. 1986) at the start of the experiment as well as the maturation stage indicate an age of 2 years for polar cod, whereas Atlantic cod were approx. 1 year old. Thus the comparison of growth rates and further characteristics of the two species in our experiment may be affected by their slightly different positions in the life cycle. The slow growth rate of polar cod (potentially amplified by low-frequency feeding events) likely prevented the detection of statistical differences in body weight increments between temperature treatments. However, due to a trend for increasing growth rates from 0 to 6 °C under control PCO_2 conditions, we assume a growth

optimum between 3 and 6 °C that coincides with the temperature preference of this species (Schurmann and Christiansen 1994). At 0 °C/390 µatm, we recorded a specific growth rate of 0.39 % d^{-1}, in line with the findings by Christiansen (1995) for polar cod from the Pechora Sea. The initial average weight as well as the feeding frequency was identical in both Christiansen's and our study. In contrast, Hop et al. (1997a) found a relative growth rate (~0.7 % d^{-1}) at 0 °C for polar cod from Canadian waters, slightly exceeding the one in our study (0.58 % d^{-1}, according to the formula in Hop et al. 1997a) despite similar initial body weight. Divergent growth rates relate to the substantially higher daily energy uptake measured by Hop et al. (1997a) (2.058 kJ d^{-1}) compared to the present study (1.436 kJ d^{-1}). Although lower feeding frequencies may have prevented individuals from exploiting their maximum growth capacity in this study, positive growth clearly demonstrates that the feed intake was sufficient to cover more than baseline costs. It remains to be explored whether growth rates differ between various polar cod populations across the Arctic as well as between fjord and oceanic stocks (Madsen et al. 2015), in similar ways as they differ between Atlantic cod populations (Pörtner et al. 2008).

A depressed feed intake of polar cod at 0 °C compared to the other experimental temperatures likely contributes to their lower growth rates at cold temperature (Drinkwater 2005). The limits of voluntary feed intake are mirrored by temperature-dependent stomach evacuation rates (Brett 1979; Hop and Tonn 1998). Low temperatures are associated with low stomach evacuation rates (evacuation half times at -1.42 ± 0.07 °C = 36–70 h) (Hop and Tonn 1998), high assimilation rates, and low energetic cost of processes competing with growth, resulting in an enhanced feed conversion efficiency at low feed intake. Hence, the thermal optimum for feed conversion results lower than the thermal optimum for growth. Árnason et al. (2009) detected a difference of 3 °C between these two optima for turbot (*Scophthalmus maximus*), while our data indicate a difference of as large as 3–6 °C for polar cod. Although digestion rates, and thus appetite, are known to rise with increasing temperatures (Brett 1979), the daily feed intake reached a plateau at all temperatures investigated above 0 °C, indicating that 18.8 mg (g wet BW*meal)$^{-1}$ may represent maximal stomach filling in polar cod from Kongsfjorden (average BW = 23.3 g). This hypothesis implies complete stomach emptying between feeding events above 0 °C; however, stomach inspection confirmed incomplete emptying at the end of the experiment. Based on identical amounts of consumed feed per meal for polar cod at 3–8 °C and enhanced metabolic rates at 8 °C, we conclude that elevated baseline costs were the constraining factor for both growth and feed conversion at 8 °C. Furthermore, faster stomach evacuation rates at 8 °C and accordingly shortened assimilation periods potentially caused decreasing exploitation efficiency of consumed feed in similar ways as described for brown trout (*Salmo trutta*) by Elliott (1976).

Another parameter interfering with somatic growth is gonadal development. In late September, male polar cod kept at 0 °C developed a mean GSI of 7.31 %, whereas no such trend was visible for the females at the same temperature (mean GSI = 2.09 %). Hop et al. (1995) reported that gonadal development in male polar cod from the Canadian Arctic maintained at 1 °C started in August and reached GSI values similar to those in our fish in September. Reproducing males exhibited a GSI of approx. 30 % from December onwards (ibid.). Females show a slow initial increase in gonadal tissue and an exponential gonadal development with a peak in gonad size approximately 2 months after males are ready to spawn (Hop et al. 1995). However, a substantial difference in specific growth rates between sexes before the winter months was not found (Christiansen 1995). The variability in GSI between temperature treatments as well as the mean GSI decreased with increasing temperatures, indicating an unfavorable shift in the energy budget. This trend is also reflected in a continuous decrease in the condition factor at higher temperatures. The lipids needed for gonadal development are very likely mobilized from liver tissue. Therefore, the increasing trend of HSI with temperature might be attributed to a decreasing ability to exploit liver energy reserves in the warmth. Furthermore, gonad development might cause a slight underestimation of growth rate at 0 °C, considering the lower weight of gonadal tissue compared to muscle tissue of identical energy content.

In contrast to the findings for polar cod, mortality among Atlantic cod occurred predominantly at an early stage of the experiment, mainly due to the total refusal of feed, followed by a few occasional deaths until the end of the incubation. As mortality was scattered across treatments, neither temperature nor PCO_2 seemed to be the cause for the recorded mortality.

The growth rates of Atlantic cod were directly correlated with temperature and no distinct thermal optimum was found within the investigated temperature range (3–16 °C). Growth optima are size- (Björnsson et al. 2007) and ratio-dependent (Brett 1979) and differ slightly between populations (Pörtner et al. 2001, 2008). Björnsson et al. (2007) found an optimum temperature for growth in similar sized (56.9 g) Islandic cod at 12.1 °C. The low frequency of feeding events may have caused a feed limitation during our experiments with potential consequences for the thermal optimum for growth. According to Brett (1979), the optimum temperature for growth shifts to lower temperatures under restricted food supply. We therefore conclude

that the temperature optimum for growth for the investigated population of Atlantic cod in the present study is unlikely above 16 °C. The limited scope for locomotion for individuals in the present study may have modified the priorities in energy allocation such that growth takes priority, especially at high temperatures where activity is known to be most pronounced and costly (e.g. Brown et al. 1989). Investigations of spontaneous activity in our fish revealed no significant differences between the temperatures (pers. obs.) supporting the hypothesis of shifted priorities in energy allocation in favor of growth. Nevertheless, Björnsson et al. (2007) recorded an optimum growth rate of 1.75 % d^{-1} (12.1 °C), which exceeds the rate in the present study (0.82 % d^{-1}, 12 °C) more than twofold for cod of similar size. Björnsson et al. (2007) fed their fish several times per day, potentially explaining the difference in growth rates. Despite the potential feed limitation in our experiment, we recorded similar daily feed consumption rates compared to a study with higher feeding frequencies: Peck et al. (2003) detected a daily feed intake of 1.62 % (wet BW*day)$^{-1}$ for Atlantic cod of 7.57 g at 12 °C fed one to three times per day with artificial diet (19.84 kJ (g wet wt.)$^{-1}$). The fish in the present study consumed 1.44 % (wet BW*day)$^{-1}$ (15.865 kJ (g wet wt.)$^{-1}$) at the same temperature and a mean body weight of 40.4 g. Although the percent feed consumption is considered to decrease with increasing body weight, the comparable amounts consumed in both studies indicate a compensation for low feeding frequency by high feed consumption per meal. High feed consumption is supported by complete stomach emptying between the feeding events at this temperature as confirmed by stomach inspections at the end of the experiment. Despite higher rates of stomach emptying (Tyler 1970) and rising metabolic costs with increasing temperatures, both condition factor and HSI increased continuously, indicating an energy surplus. Accordingly, feeding was sufficient to support positive growth at all temperatures. Along similar lines of reasoning, the increasing feed consumption rates per unit body weight at higher temperatures were likely sufficient to cover the higher maintenance costs, indicated by constant feed conversion efficiencies at increasing temperatures. Solely at 3 °C, the feed conversion efficiency appeared to be reduced [by 34.2 % (390 µatm) and 30.6 % (1170 µatm)]. At this temperature, the feed intake is very likely limited by low stomach evacuation rates and low digestion rates. Accordingly, 3 °C approaches the lower thermal limit of this species' metabolic efficiency, potentially evoked by insufficient capacity of the digestive system (Pörtner 2001). However, cod are still able to exploit this temperature range as the habitat temperatures experienced by adult Atlantic cod from the Barents Sea were found to fall temporarily below 3 °C (Michalsen et al. 2014). At all comparable temperatures, Björnsson et al. (2001) found higher feed conversion efficiencies for Atlantic cod than recorded in this study, possibly indicating a high plasticity of this species to adapt to different feeding regimes.

Further considerations include a potential functional and genetic difference between different stocks of Atlantic cod. The fish in Björnsson et al. (2007) were hatchery reared and originated from an Icelandic broodstock, while our fish were caught in the area around Svalbard. Growth performance of different cod stocks has been shown to decrease with increasing latitude (Fischer 2002). The selection of specific phenotypes from the larval population at high mortality may cause such functional differences. Although genetic differences in growth performance cannot be eliminated by acclimatization processes (Pörtner et al. 2001), the differences in growth rates of different populations reared under similar conditions appear to be small (Purchase and Brown 2001, cf. Pörtner et al. 2008). Therefore, Björnsson and Steinarsson (2002) considered a genetic contribution to Atlantic cod growth to be less than the influence of environmental factors.

Although the specimens of both species originate from an environment with temperatures well below their optimum for growth, the stenothermal polar cod is very well adapted to the low temperatures, whereas the eurythermal Atlantic cod appears to reach the lower boundaries of its thermal window. Despite higher maximal growth capacity in Atlantic than in polar cod, the better growth performance at low temperatures emphasizes the higher degree of cold adaptation of polar cod than of Atlantic cod coexisting in the same area. Consequently, the majority of polar cod in the present study were caught at temperatures between −1.5 and 3.0 °C in bottom waters, whereas juvenile Atlantic cod were caught in surface waters at temperatures between 4.0 and 6.0 °C. Hence, the benefit of low predation pressure for polar cod at low temperatures (Brown et al. 1989) seems to outweigh the physiological restrictions. The high feed conversion efficiency at low temperatures enables polar cod to reveal positive growth even with little or temporally restricted food supply. Furthermore, reduced foraging activity involves less exposure to potential predators. In contrast to polar cod, Atlantic cod is well adapted to handle high food abundances (Jensen et al. 1991), indicated by greatly varying feed consumption rates with temperature. Therefore, its growth performance, as well as the temperature optimum for growth in nature, are strongly correlated with food availability (Jobling 1994). The physiology of Atlantic cod seems to be specialized in efficient and fast growth in order to reduce predation pressure at small body size. The feed conversion efficiency of Atlantic cod decreases remarkably in the cold, evoked by a stronger impairment of feed intake compared to the

decrease in maintenance costs. Nevertheless, adult Atlantic cod can experience and tolerate subzero temperatures for short periods (Michalsen et al. 2014), making them likely candidates to prey upon polar cod in deep, cold water layers in the fjords around Svalbard.

Under projected climate conditions for the year 2100, growth performance of polar cod is expected to remain unchanged. However, from the present perspective, its feed conversion efficiency can be assumed to decrease progressively. Considering that other temperate fish species are also extending their distribution range to the North, such competition might cause a northward displacement of polar cod. Hop and Gjøsæter (2013) expect the pelagic capelin (*Mallotus villosus*) and herring (*Clupea harengus*) as likely invader species replacing polar cod as a key species in the area of investigation. Atlantic cod is expected to thrive under future ocean conditions in the area around Svalbard, provided that its prey species also adapt to the environmental changes or that it is able to switch the species preyed upon. However, the distribution of demersal Atlantic cod is likely constrained by the shelf edge (Hop and Gjøsæter 2013), whereas ice-associated polar cod is found throughout the Arctic Ocean. Ultimately, it is not the increase in annual mean temperature but rather the change in extreme temperatures that will be crucial for a species' settlement (Stachowicz et al. 2002).

In conclusion, the combined drivers ocean acidification and warming (OAW) caused nonsignificant trends in growth performance and SMR of polar cod and in feed consumption and HSI of Atlantic cod. While the performance of polar cod will be impaired under projected water conditions by year 2100, Atlantic cod will thrive, visible in lower amounts of feed necessary for the maintenance of growth performance. The higher tolerance of Atlantic cod to changing water conditions is likely attributed to the wide range of abiotic habitat conditions experienced within its lifetime. Temperature is the predominant environmental factor causing the shift in relative performance by influencing the energy allocation between individual processes in the energy budget of both species. The stenothermal polar cod is specialized in temperatures well below its thermal optimum for growth and can thrive with little food supply, thereby escaping competitive pressure. Atlantic cod is eurythermal and rather displays high plasticity under changing abiotic conditions, but is less competitive in the cold. Hence, we argue that the competitive strength of polar cod is expected to decrease dramatically under future warming and acidification. Therefore, a northward displacement and thereby a decreasing distribution range of polar cod will be the likely consequence of the ongoing distribution shift of Atlantic cod to the north. However, the reported effects may become less pronounced over time depending on potential trans-generational adaptation effects.

Acknowledgments This project was funded through the research program BIOACID (Biological Impacts of Ocean Acidification, phase II) by the German Federal Ministry of Education and Research (BMBF, WP 4.1 and 4.2, FKZ 03F0655B, FKZ 03F0728B). All authors acknowledge funding through the PACES (Polar Regions and Coasts in a Changing Earth System) program of the Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI). Furthermore, the authors gratefully acknowledge Jasmine Nahrgang and the project Polarisation (Norwegian Research Council, No. 214184/F20) for providing polar cod. We thank the crews of RV Heincke (AWI, funding No. AWI_HE 408_00) and RV Helmer Hanssen (University of Tromsø) for animal collection. Further, we would like to thank Timo Hirse and Sebastian Berger for technical assistance with the manipulation of CO_2 partial pressure, Anette Tillmann, Karim Zanaty, Marcel Machnik, Benjamin Matthei and Fredy Véliz Moraleda for their contribution to the measurements of pH and DIC, and Christiane Hassenrück for determining the stomach weights of polar cod. We highly appreciate the constructive comments of the editor Dieter Piepenburg, Tony Hickey, Harald Gjøsæter and one anonymous referee on the submitted manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in the present study were in accordance with the ethical standards of the federal state of Bremen, Germany, and were approved under the reference number 522-27-22/02-00 (113).

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PUBLICATION II

Aerobic capacities and swimming performance of Polar cod (*Boreogadus saida*) under ocean acidification and warming conditions

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2018

Journal of Experimental Biology, 221(21), 1-11

submitted: 10 May 2018
accepted: 1 September 2018
published: 31 October 2018
doi: 10.1242/jeb.184473

http://jeb.biologists.org/content/221/21/jeb184473.abstract

© 2018. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2018) 221, jeb184473. doi:10.1242/jeb.184473

RESEARCH ARTICLE

Aerobic capacities and swimming performance of polar cod (*Boreogadus saida*) under ocean acidification and warming conditions

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ABSTRACT

Polar cod (*Boreogadus saida*) is an important prey species in the Arctic ecosystem, yet its habitat is changing rapidly: climate change, through rising seawater temperatures and CO_2 concentrations, is projected to be most pronounced in Arctic waters. This study aimed to investigate the influence of ocean acidification and warming on maximum performance parameters of *B. saida* as indicators for the species' acclimation capacities under environmental conditions projected for the end of this century. After 4 months at four acclimation temperatures (0, 3, 6, 8°C) each combined with two P_{CO_2} levels (390 and 1170 µatm), aerobic capacities and swimming performance of *B. saida* were recorded following a U_{crit} protocol. At both CO_2 levels, standard metabolic rate (SMR) was elevated at the highest acclimation temperature indicating thermal limitations. Maximum metabolic rate (MMR) increased continuously with temperature, suggesting an optimum temperature for aerobic scope for exercise (AS_{ex}) at 6°C. Aerobic swimming performance (U_{gait}) increased with acclimation temperature irrespective of CO_2 levels, while critical swimming speed (U_{crit}) did not reveal any clear trend with temperature. Hypercapnia evoked an increase in MMR (and thereby AS_{ex}). However, swimming performance (both U_{gait} and U_{crit}) was impaired under elevated near-future P_{CO_2} conditions, indicating reduced efficiencies of oxygen turnover. The contribution of anaerobic metabolism to swimming performance was very low overall, and further reduced under hypercapnia. Our results revealed high sensitivities of maximum performance parameters (MMR, U_{gait}, U_{crit}) of *B. saida* to ocean acidification. Impaired swimming capacity under ocean acidification may reflect reduced future competitive strength of *B. saida*.

KEY WORDS: Climate change, Gadids, Arctic cod, Hypercapnia, RCP8.5, Aerobic scope

INTRODUCTION

The oceans are currently experiencing a warming trend in parallel with increasing P_{CO_2} levels (Caldeira and Wickett, 2003; IPCC, 2014). These changes are expected to be fastest in Arctic waters due to the high solubility of CO_2 in cold waters (Fransson et al., 2009), and an increase in the temperature of Atlantic water masses flowing into the Arctic ocean (Polyakov et al., 2010). This accelerates the decline in sea-ice cover and the freshening of surface waters (McPhee et al., 1998), which, in turn, exacerbates ocean acidification due to decreasing buffer capacities (Steinacher et al., 2009). According to the Representative Concentration Pathway representing business-as-usual CO_2 emissions (RCP 8.5), the Arctic is projected to experience a rise in surface temperatures of 4–11°C by the year 2100 compared with the period 1986–2005 (IPCC, 2014). Within the same timeframe, P_{CO_2} levels in the Arctic ocean are projected to rise from 400 µatm to up to 1370 µatm (IPCC, 2014). Warming and potentially other climate change stressors such as ocean acidification appear to be already causing large-scale geographic shifts of marine species (Poloczanska et al., 2014) such as the ongoing borealization of the Arctic (Fossheim et al., 2015), entailing significant effects on the Arctic food chain.

Temperature is considered to be the most important abiotic factor shaping the geographical distribution of aquatic species (Magnuson et al., 1979; Perry et al., 2005; Fossheim et al., 2015) because of its effects on biochemical and physiological processes (Reynolds and Casterlin, 1979; Pörtner and Farrell, 2008). Accordingly, ectotherms tolerate a range of species-specific habitat temperatures that support the functionality of their molecular, cellular and systemic processes (Pörtner and Farrell, 2008). The species' thermal performance window can be understood from the ability of aerobic metabolic capacities to cover higher-than-baseline maintenance costs (Pörtner, 2010), as exemplified by aerobic scope (AS) [maximum metabolic rate (MMR)−standard metabolic rate (SMR)]. Within the thermal window, aerobic scope increases towards a species-specific optimum temperature and decreases rapidly at thermal conditions exceeding the optimum (Pörtner and Farrell, 2008; Farrell, 2016; Pörtner et al., 2017). Thermal performance windows are delimited by upper and lower critical temperatures at which aerobic scope reaches zero, solely supporting a time-limited passive and anaerobic existence (Pörtner and Farrell, 2008). In contrast, maximized aerobic scope at the species-specific optimum temperature implies optimum conditions for the performance of a given activity (Fry, 1947). Assuming that different aerobic activities do not necessarily have identical optimum temperatures, a broad thermal window with maximum aerobic scope covering a wide thermal range implies reduced competition between aerobic activities (Farrell, 2016). Most polar species, however, are adapted and specialized to the low temperatures and low thermal fluctuations of their natural habitat by evolving mechanisms to maintain overall performance along with reduced tolerance to changing abiotic conditions as a trade-off (Pörtner et al., 2000, 2005). Even relatively small increments in

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Received 10 May 2018; Accepted 1 September 2018

Journal of Experimental Biology

RESEARCH ARTICLE Journal of Experimental Biology (2018) 221, jeb184473. doi:10.1242/jeb.184473

List of abbreviations and symbols

AS_{ex}	aerobic scope of exercise
BC_{max}	maximum burst count
BC_{tot}	total number of bursts
BL	body length
BW	body weight
E_{max}	efficiency of maximum swimming performance
$\dot{M}_{O_2}$	rate of oxygen consumption
MMR	maximum metabolic rate
OAW	ocean acidification and warming
P_{CO_2}	partial pressure of carbon dioxide
pH_{tot}	total pH
SMR	standard metabolic rate
$T_{C,max}$	critical thermal limit
T_{pej}	pejus temperature
TSB	time between U_{gait} and U_{crit} ('time spent bursting')
$TSB_{anaerob}$	estimated proportion of anaerobic metabolism between U_{gait} and U_{crit}
U	swimming speed
U_{crit}	maximum swimming speed
U_{gait}	transition speed from purely aerobic to partly anaerobic swimming (~maximum aerobic swimming speed)
U_{max}	highest speed maintained for full time interval
v	velocity increment

temperature can therefore have a tremendous impact on their metabolic demand (Claireaux et al., 2000; Pörtner, 2010) entailing a rise in energy turnover with detrimental consequences for fitness and performance traits e.g. growth, reproduction and swimming capacity (Butler et al., 1992). Despite decreasing performance capacities, the thermal range between peak aerobic scope and upper critical temperature is considered to be a buffer against a future increase in water temperature (Farrell, 2016), for the case where northward distribution shifts triggered by the motivation to preserve organismic performance cannot fully compensate for ocean warming.

Polar cod (*Boreogadus saida*, Lepechin 1774), is the most abundant Arctic gadid (Mueter et al., 2016 and references therein) and it is regarded as a key species in Arctic ecosystems (Bain and Sekerak, 1978; Welch et al., 1993; Hop and Gjøsæter, 2013) because of its role as a link between lower and higher trophic levels (Lowry and Frost, 1981; Bradstreet, 1982; Welch et al., 1993). Furthermore, it is the most energy-rich prey organism in the Arctic food chain (Harter et al., 2013). In recent years, the abundance of *B. saida* has been found to decrease in its southern distribution area in the Barents Sea as a result of rising water temperatures (Eriksen et al., 2015). Throughout its life stages, *B. saida* prefers different thermal habitats. Spawning takes place in shallow waters above 0°C with a peak period in January and February (Ajiad et al., 2011). Pelagic 0-group *B. saida* prefer 2.0–5.5°C (Eriksen et al., 2015), while juveniles and non-spawning adults are either ice-associated (Lønne and Gulliksen, 1989) or found in deep water layers below 0°C (Falk-Petersen et al., 1986). A progressive distribution retreat of *B. saida*, evoked directly or indirectly by climate change, might have profound, cascading ecological consequences. In order to gauge ecosystem impacts caused by rapidly changing abiotic conditions, the assessment of sensitivities and acclimation capacities of key species such as *B. saida* to future climate scenarios is highly important.

The whole-animal SMR is an important parameter for the assessment of long-term survival because it integrates essential cellular and molecular energetic costs in the inactive organism at the respective environmental conditions (Chabot et al., 2016). Therefore, the SMR of *B. saida* has been identified over a range of acclimation temperatures in a number of studies (Holeton, 1974; Steffensen et al., 1994; Hop and Graham, 1995; Kunz et al., 2016a). Maximum respiratory performance of *B. saida* has been studied by Drost et al. (2016); however, aerobic swimming capacity as a fitness parameter has never been quantified in *B. saida*.

Furthermore, studies investigating performance capacities of *B. saida* exposed to combined climate drivers such as ocean acidification and warming (OAW) are still scarce. Recent studies investigated growth performance, feed consumption and SMR (Kunz et al., 2016a), laterality and spontaneous activity (Schmidt et al., 2017) and heart mitochondria performance (Leo et al., 2017) in juveniles as well as survival rates, SMR and morphology at hatch in early life stages (Flemming Dahlke, Daniela Storch and H.-O.P., unpublished data) under combined OAW conditions. We hypothesize that traits involving maximum performance will also be affected by ocean acidification, possibly even more so than routine functions, as the former may reflect limits to acclimatization. Therefore, the aim of the present study is to investigate the impact of long-term exposure to projected OAW scenarios on AS_{ex} and swimming performance of *B. saida* in light of its whole-animal acclimation capacities to future Arctic water conditions.

MATERIALS AND METHODS

All procedures reported in the present study were in accordance with the ethical standards of the federal state of Bremen, Germany, and were approved under the reference number 522-27-22/02-00 (113).

Fish

B. saida originated from Isfjorden and Kongsfjorden on the west coast of Spitsbergen. They were caught by RV Helmer Hanssen using bottom trawls at a depth of 120 m in January 2013. A fish-lift connected to the trawl (Holst and McDonald, 2000) protected the fish from injuries during trawling. The animals were then kept in the aquaria of Havbruksstasjonen i Tromsø AS (HiT) until April 2013, when they were transported to the laboratories of the Alfred Wegener Institute (AWI) in Bremerhaven.

Experimental design

B. saida specimens were acclimated to different combinations of present and projected future ocean water conditions (temperature: 0, 3, 6, 8°C; P_{CO_2}: 390 and 1170 µatm) for approximately 4 months. Each temperature/P_{CO_2} treatment comprised 12 individuals placed in 24 litre aquaria. The P_{CO_2} conditions for each treatment were generated in a common header tank (~200 litres) which then provided identical conditions in each of the individual tanks. The setting of P_{CO_2} levels was accomplished by equilibration with mixtures of air and CO_2 provided by an automated mass flow controller system (4 and 6 channel MFC system, HTK, Hamburg, Germany).

The distribution of individuals between treatments was done randomly. The acclimation to experimental temperatures took place gradually (max. temperature change: 1°C in 24 h), followed by establishing experimental P_{CO_2} conditions within 1 day, as soon as the desired experimental temperatures were reached. Light conditions were maintained at 12 h light:12 h dark throughout the experiment. Each fish was fed *ad libitum* every fourth day with formulated high-protein feed pellets (Amber Neptun, 5 mm, Skretting AS, Norway). For details on whole-animal parameters throughout the incubation period, see Kunz et al. (2016b).

Water chemistry

Temperature, salinity and pH (cross-calibrated to total pH scale) were monitored once to twice a week in triplicate for every treatment

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in order to verify the stability of P_{CO_2} conditions, as described in Kunz et al. (2016a). The seawater carbonate chemistry was calculated in the program CO2SYS (Lewis and Wallace, 1998) based on the total dissolved inorganic carbon and the pH_{tot} values as listed in table 2 in Kunz et al. (2016a). The full water chemistry raw data of the incubation can be found in Schmidt et al. (2016).

Swimming performance measurements

Two swim tunnels (30 litres; dimension working section: 46.5×13.5×14 cm, Loligo Systems ApS, Denmark) were used simultaneously to determine the swimming performance of *B. saida* (n=4–6 per treatment), enabling the measurement of up to 6 individuals per day in temperature-controlled rooms. The swim tunnels were supplied with pre-conditioned water from the header tank of the respective incubation treatment. For the time span of the experiment, the water conditions in the tunnels were maintained by permanent aeration with a gas mixture containing the respective CO_2 levels. Aeration was maintained in the reservoir tank surrounding the swimming chamber. The swim chamber was kept in open mode to avoid decreasing O_2 concentrations as well as temperature and P_{CO_2} fluctuations in the chamber. Permanent seawater exchange between the outer reservoir tank and the swim chamber was established by an aquarium pump (9.2 litres min^{-1}). The desired velocity was translated from a control unit to a propeller in the swim chamber. A uniform velocity profile and a laminar flow were promoted by honeycomb-shaped plastic inserts. A flow sensor (Vane wheel flow sensor FA, Höntzsch Instruments, Waiblingen, Germany) placed in the centre of the working section of the swim tunnel was used to calibrate the water velocity to voltage output from the control unit.

The fish were transferred to the swim tunnel on the third day after feeding. The experiment was started after an average period of 3.5 h of animal adjustment to the system at a basic velocity of 1.4–2.2 BL s^{-1}. The swim tunnel was covered with an opaque plastic curtain in order to minimize disturbance due to movements in the room. Following the initial period of adjustment, the velocity was slowly, but continuously increased to the mean start velocity per treatment of 2.4–2.8 BL s^{-1} (with respect to different size classes at different temperatures), depending on swim tunnel and fish size, with larger fish exposed to higher starting velocities to obtain similar relative velocities (BL s^{-1}). According to the developed swim protocol, each velocity step was maintained for 11 min. At each velocity step, burst-and-coast (also known as kick-and-glide) swimming events were counted for 30 s after 5 and 10 min. This count aimed at determining the water velocity at gait transition (U_{gait}) from steady to unsteady swimming mode. Following the second counting, water velocity was slowly increased by 1.9± 0.3 cm s^{-1}. The experiment ended when the fish were exhausted, defined by their physical contact to the grid for at least 30 s. The critical swimming speed (U_{crit}) was adjusted according to the actual time spent at the maximal velocity as suggested by Brett (1964):

$$U_{crit} = U_{max} + \frac{vT}{t}, \quad (1)$$

where U_{max} is highest velocity maintained for full time interval, v is velocity increment, T is time spent at the velocity leading to fatigue and t is time interval. As soon as the fish gave up swimming, the velocity was rapidly decreased to the basic weaning velocity and fish were immediately transferred into respiration chambers. The short period of air exposure was used to weigh the fish to the nearest 0.1 g.

Respiration measurements

Individual rates of oxygen consumption ($\dot{M}_{O_2}$ in µmol min^{-1} g^{-1}) were measured at long-term acclimation temperature and P_{CO_2} by automated intermittent flow-through respirometry in a separate experimental set-up, comprising two sets of 6 perspex respiration chambers (1.8 and 2.2 litres). Respiration chambers were submerged as sets of two in common tanks (~50 litres) with water conditions identical to the respective temperature/P_{CO_2} treatments. Partial water exchanges with pre-conditioned sea water were performed after ~24 h. A non-transparent plastic wall between the respiration chambers prevented visual contact of the two individuals sharing a common water basin. Aeration of the water surrounding the respiration chambers with the respective air/CO_2 mix was maintained throughout the experimental period to ensure oxygen saturation.

The water inside the respiration chambers circulated permanently at constant velocity by aid of an aquarium pump (8.2 litres min^{-1}). A flush pump (5.0 litres min^{-1}) facilitated periodic water exchanges between respirometer and its surrounding. $\dot{M}_{O_2}$ measurement periods of 15 min were alternated with flush periods of 30 min to fully re-establish O_2 saturation. The O_2 concentration was determined by optical oxygen probes and recorded using a ten-channel oxygen meter (PreSens-Precision Sensing GmbH, Hamburg, Germany; system 1) as well as a four-channel FireStingO2 (Pyro Science GmbH, Aachen, Germany) and two one-channel Fibox 3 systems (PreSens-Precision Sensing GmbH, Hamburg, Germany) (system 2). For the 0% calibration, the oxygen probes were flushed with nitrogen at room temperature. The calibration for 100% O_2 was performed in fully aerated water at the respective experimental temperature prior to the measurements of each treatment. Blank measurements to detect bacterial background respiration were recorded following the $\dot{M}_{O_2}$ analyses once at every temperature. In order to minimize potential disturbances, all tanks were covered with opaque plastic sheets.

In the respiration chambers, both MMR and SMR were determined at long-term acclimation temperature and P_{CO_2}. To obtain SMR, individuals remained in the chambers for ~48 h in order to fully recover from exercise in the swim tunnel. The $\dot{M}_{O_2}$ values were calculated using the appropriate constants for O_2 solubility in seawater (Boutilier et al., 1984) and normalized to an average fish weight of 25.6 g following Steffensen et al. (1994). After subtraction of bacterial respiration (solely measurable at 8°C), the first 5 min of the slope of the first $\dot{M}_{O_2}$ recording were used to calculate MMR, while the 15% quantile of $\dot{M}_{O_2}$ recordings starting from the second night in the respiration chamber was considered as SMR (Chabot et al., 2016). After respiration measurements, the length of each fish was measured.

Calculations and statistical analysis

$\dot{M}_{O_2}$ data were normalized to an average fish mass (25.6 g) according to Steffensen et al. (1994):

$$\dot{M}_{O_2(25.6)} = \dot{M}_{O_2}\left(\frac{BW}{25.6}\right)^{(1-0.8)}. \quad (2)$$

Aerobic scope for exercise (AS_{ex}) was defined as:

$$AS_{ex} = MMR - SMR. \quad (3)$$

The index of the energetic efficiency of maximum swimming performance (E_{max}) was calculated as the ratio U_{crit} MMR^{-1}. Based on concerns outlined by Brett (1962), this index assumes that a

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potential oxygen debt accumulated during exhaustive burst-type exercise is negligible.

The contribution of anaerobic metabolism (%) during the period between U_{gait} and U_{crit} was approximated using a duration of one second per burst:

$$TSB_{anaerob} = \frac{BC_{tot} \times 100}{TSB}, \quad (4)$$

where TSB is time spent bursting (time between U_{gait} and U_{crit} in s), BC_{tot} is total number of bursts, $TSB_{anaerob}$ is the estimated proportion of anaerobic metabolism between U_{gait} and U_{crit}.

In order to further classify anaerobic swimming performance, we analysed both the maximum consecutive number of bursts at one velocity step and the total number of bursts throughout the whole swim trial.

Individuals that displayed physical abnormalities (n=1; 0°C/1170 µatm) or refused to swim (n=2; 0°C/390 µatm, 8°C/1170 µatm) because of lethargic behaviour were excluded from data analysis. Fish that refused to swim for no apparent reason (n=1; 6°C/1170 µatm) were included in the analysis for SMR. Individuals that did not have any burst capacity were excluded from the statistical analysis for U_{crit} for comparability reasons.

Statistical analyses were accomplished using R version 3.0.2 (2013). All variables were tested for normal distribution and homoscedasticity with Shapiro–Wilk and Levene tests, respectively. Owing to heteroscedasticity, the data sets for BC_{tot} and $TSB_{anaerob}$ were log and square root transformed, respectively. Following Nalimov tests, one outlier was removed from the variable AS_{ex} (3°C/390 µatm; P=0.0111), U_{gait} (3°C/1170 µatm; P=0.0007), E_{max} (0°C/390 µatm; P=0.0073) and $TSB_{anaerob}$ (0°C/390 µatm; P=0.0241), respectively. Outlier tests proved inefficient within the one treatment of the variable BC_{tot} (0°C/390 µatm, P=0.0233). Therefore, this treatment was tolerated as false positive during further statistical analysis. Statistical comparisons between treatments were performed for the variables SMR, MMR, AS_{ex}, U_{gait}, U_{crit}, E_{max}, maximum burst count (BC_{max}), BC_{tot}, TSB and $TSB_{anaerob}$ using two-way ANOVA. In the case of statistically significant differences, a subsequent *post hoc* Tukey honest significance test was applied. The results of the two-way ANOVA are shown in Table 1, while the results of the Tukey honest significance test between temperature treatments are shown as letters within the figures. Significant differences were assumed using a 5% threshold (P<0.05).

The mean number of bursts was expected to increase exponentially with swimming speed. Therefore, we used SigmaPlot 13 (Systat Software Inc., San Jose, California, USA) to find an exponential model with the best fit. The general relationship between swimming speed and burst number at 0, 3 and 8°C was described best by the following model:

$$\text{Mean burst count } (U) = a \times \exp^{(b \times U)}. \quad (5)$$

This model was also applied to the data set of 6°C/390 µatm, although the model fit was relatively poor for the data of this treatment (see Table 2 for significance levels). Significant differences in the burst performance between P_{CO_2} treatments were accepted in the case of non-overlapping 95% confidence intervals.

RESULTS

Table 3 provides a summary of the results for respiration measurements and swimming performance. A summary of burst swimming parameters is given in Table 4. In total, four individuals showed no bursting event (6°C/390 µatm, n=1; 6°C/1170 µatm, n=1; 8°C/390 µatm, n=2). For a further three individuals (0°C/390 µatm, n=1; 6°C/390 µatm, n=1; 6°C/1170 µatm, n=1), bursting occurred very close to the critical swimming speed (U_{crit}).

Respiration

The SMR of *B. saida* showed comparable values at 0, 3 and 6°C, but was significantly higher at 8°C (0°C versus 8°C, P<0.0001; 3°C versus 8°C, P<0.0001; 6°C versus 8°C, P=0.0005). Hypercapnia did not reveal any effect on the SMR of this species (P=0.342) (Fig. 1A). Long-term acclimation to different temperatures had a distinct effect (P=0.0005) on the MMR of *B. saida*: MMR rose significantly between 0 and 6°C (P=0.0041), where it levelled off (6°C versus 8°C, P=0.9232). At all temperatures but 0°C, MMR was enhanced in high P_{CO_2} treatments compared with control P_{CO_2} treatments (P=0.0322) (Fig. 1B). An overall temperature effect (P=0.0185) was recorded for the aerobic scope of exercise (AS_{ex}) after 4 months with a peak observed at 6°C (0°C versus 6°C, P=0.0336). Furthermore, AS_{ex} was significantly elevated under high P_{CO_2} conditions (P=0.0059) (Fig. 2).

Swimming performance

The transition speed from purely aerobic to partly anaerobic swimming performance (U_{gait}) increased significantly with long-term acclimation temperature (P=0.0341). The significant difference, nevertheless, refers to an elevated U_{gait} at the highest (8°C) compared with the lowest acclimation temperature (0°C), indicating an overall modest temperature effect on this parameter. Long-term acclimation at high P_{CO_2} significantly depressed U_{gait} (P=0.0270; Fig. 3A). An interaction between temperature and P_{CO_2} level was not found for this parameter (P=0.8134). U_{crit} did not

Table 1. Results of two-way ANOVA

	Temperature effect			P_{CO_2} effect			Interaction effect		
	F	d.f.	*P*	*F*	d.f.	*P*	*F*	d.f.	*P*
SMR	12.044	3	**<0.0001**	0.927	1	0.343	0.863	3	0.470
MMR	7.870	3	**0.0005**	5.048	1	**0.0322**	1.415	3	0.2577
AS_{ex}*	3.955	3	**0.0185**	8.928	1	**0.0059**	2.103	3	0.1233
U_{gait}*	3.303	3	**0.0341**	5.423	1	**0.0270**	0.316	3	0.8134
U_{crit}	1.626	3	0.2014	3.920	1	0.0559	0.293	3	0.8299
E_{max}*	4.821	3	**0.0085**	2.831	1	0.1045	3.047	3	**0.0465**
BC_{max}	4.707	3	**0.0075**	5.664	1	**0.0231**	1.101	3	0.3623
BC_{tot}‡	2.439	3	0.0838	3.220	1	0.0828	3.453	3	**0.0288**
TSB	4.951	3	**0.0059**	0.025	1	0.8760	0.373	3	0.7733
$TSB_{anaerob}$*,§	2.394	3	0.086	0.406	1	0.529	0.301	3	0.824

Significant *P*-values are shown in bold. *One outlier was excluded; ‡data set log-transformed; §data set square root-transformed.

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Table 2. Significance levels (adjusted R^2) for the exponential model describing the increase in the mean number of bursts with swimming speed separated by treatment

	Temperature			
	0°C	3°C	6°C	8°C
390 µatm CO_2	0.7837 (*n*=5)	0.6405 (*n*=6)	0.0418 (*n*=5)	0.4649 (*n*=6)
1170 µatm CO_2	0.5464 (*n*=5)	0.8465 (*n*=6)	0.3766 (*n*=5)	0.2710 (*n*=4)

Number in brackets is number of investigated specimens.

reveal a significant temperature effect (P=0.2014). U_{crit} data, however, indicated a downward trend due to hypercapnia (P=0.0559; Fig. 3B).

The maximum number of bursts, a parameter assumed to reflect the capacity for anaerobic swimming, was highest at 3°C (0°C versus 3°C, P=0.1358; 3°C versus 6°C, P=0.0051; 3°C versus 8°C, P=0.0776). At the same time, the maximum burst count was found to be higher under normocapnia than hypercapnia (P=0.0231). Furthermore, elevated P_{CO_2} shifted mean burst performance at 3°C to lower velocities under hypercapnia (non-overlapping 95% CI; asterisk in Fig. 4). In contrast to the results for maximum number of bursts, neither temperature nor P_{CO_2} affected the total number of bursts significantly. However, a combined effect of temperature and P_{CO_2} level was detected (P=0.0288), mainly evoked by the low number of bursts detected in the treatment at 8°C/1170 µatm. The time between U_{gait} and U_{crit} – hereafter classified as 'time spent bursting' (TSB) – revealed a decreasing trend with temperature (P=0.0059), with no apparent P_{CO_2} effect (P=0.8760). The putative contribution of anaerobic metabolism to swimming performance between U_{gait} and U_{crit} ($TSB_{anaerob}$) was low overall (<3% in 92.9% of individuals) with no apparent influence of temperature or P_{CO_2} (Table 4).

Energetic efficiency of maximum swimming performance (E_{max}) (Fig. 5) was high at 0°C (0°C versus 3°C, P=0.1161; 0°C versus 6°C, P=0.0128; 0°C versus 8°C, P=0.0210). Although no significant impact of hypercapnia was detected (P=0.1045), E_{max} was reduced under high P_{CO_2} conditions at all temperatures above 0°C due to the elevated MMR under hypercapnic conditions between 3 and 8°C. Furthermore, E_{max} showed a significant interaction effect of temperature and P_{CO_2} evoked by a strongly elevated value at 0°C under hypercapnia (0°C/1170 µatm versus 3°C/1170 µatm, P=0.0236; 0°C/1170 µatm versus 6°C/1170 µatm, P=0.0348; 0°C/1170 µatm versus 8°C/1170 µatm, P=0.0155).

DISCUSSION

The present study aimed to investigate oxygen consumption and exercise capacities of *B. saida* after long-term acclimation to future OAW conditions in order to estimate the competitive strength of this species under future environmental conditions at P_{CO_2} levels following the RCP8.5 scenario (IPCC, 2014). Our results suggest that enhanced costs visible in elevated MMR under hypercapnic water conditions cause a reduction in maximum swimming capacity.

At comparable temperatures, the SMR obtained in the present study was in the same order of magnitude as published for *B. saida* (Steffensen et al., 1994, 4.5°C; Drost et al., 2016, 1.0, 3.5, 6.5°C), when applying the $\dot{M}_{O_2}$ units and the weight correction formula of the respective studies. Slight deviations in SMR are likely to be attributable to divergent approaches to determine SMR: when applying the same approach for the determination of SMR as used by Drost et al. (2016) (assuming the lowest $\dot{M}_{O_2}$ recording as SMR) for comparison between both studies, the resulting SMR values of the present study (65, 64 and 78 mg O_2 kg^{-1} h^{-1} at 0, 3 and 6°C, respectively) are fairly similar to those published by Drost et al. (2016) (~53, 50 and 76 mg O_2 kg^{-1} h^{-1} at 1, 3.5 and 6.5°C, respectively). The approach chosen by Drost et al. (2016) was the only method applicable to their particular experimental design. However, Chabot et al. (2016) raised the concern of an underestimation of SMR due to temporal variability within this parameter when only a single $\dot{M}_{O_2}$ measurement is chosen to represent SMR. In order to correct for temporal variability, we preferred to calculate SMR by aid of a quantile approach allowing

Table 3. Summary of respiration measurements and swimming performance results

		Temperature			
		0°C	3°C	6°C	8°C
390 µatm CO_2	Wet weight (g)	23.3±1.9 (5)	26.8±4.7 (6)	26.4±1.7 (6)	20.5±2.7 (6)
	Total length (cm)	14.9±0.4 (5)	15.2±0.8 (6)	15.6±0.2 (6)	14.8±0.7 (6)
	SMR (µmol min^{-1} g^{-1})	0.0537±0.0030 (5)	0.0503±0.0042 (4)	0.0592±0.0030 (5)	0.0838±0.0074 (5)
	MMR (µmol min^{-1} g^{-1})	0.0970±0.0082 (5)	0.0995±0.0198 (4)	0.1249±0.0033 (5)	0.1333±0.0102 (5)
	AS_{ex} (µmol min^{-1} g^{-1})	0.0432±0.0088 (5)	0.0305±0.0020 (3)	0.0649±0.0061 (4)	0.0495±0.0083 (5)
	U_{gait} (BL s^{-1})	3.04±0.19 (5)	3.27±0.12 (6)	3.30±0.24 (4)*	3.63±0.19 (4)*
	U_{crit} (BL s^{-1})	3.49±0.19 (5)	3.51±0.16 (6)	3.37±0.23 (4)‡	3.88±0.12 (4)‡
	E_{max} (BL g $µmol^{-1}$)	1968.1±51.9 (4)	2187.4±333.3 (4)	1600.5±85.2 (4)	1799.7±235.3 (4)
1170 µatm CO_2	Wet weight (g)	21.8±2.2 (5)	34.3±4.0 (6)	32.3±2.6 (6)	19.5±0.9 (4)
	Total length (cm)	14.4±0.3 (5)	16.9±0.5 (6)	17.0±0.5 (6)	14.7±0.4 (4)
	SMR (µmol min^{-1} g^{-1})	0.0505±0.0042 (5)	0.0525±0.0060 (6)	0.0589±0.0029 (6)	0.0708±0.0059 (4)
	MMR (µmol min^{-1} g^{-1})	0.0870±0.0106 (5)	0.1319±0.0157 (6)	0.1545±0.0049 (4)	0.1618±0.0153 (4)
	AS_{ex} (µmol min^{-1} g^{-1})	0.0364±0.0126 (5)	0.0778±0.0172 (5)	0.0945±0.0061 (4)	0.0911±0.0196 (4)
	U_{gait} (BL s^{-1})	2.94±0.09 (5)	2.95±0.02 (5)	3.11±0.15 (4)*	3.28±0.10 (4)
	U_{crit} (BL s^{-1})	3.32±0.14 (5)	3.06±0.15 (6)	3.29±0.21 (4)‡	3.42±0.15 (4)
	E_{max} (BL g $µmol^{-1}$)	2444.0±352.0 (5)	1460.0±121.1 (6)	1312.0±71.1 (3)	1300.4±121.9 (4)

All values are means±s.e.m. Number of individuals per variable is shown in brackets. *Single individuals not showing burst behaviour.‡U_{crit} of individuals without burst behaviour were not included in the mean, because the respective values were also excluded from data analysis.

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Table 4. Summary of anaerobic swimming parameters

		Temperature			
		0°C	3°C	6°C	8°C
390 µatm CO_2	BC_{max}	11.4±3.4 (5)	19.5±3.4 (6)	5.2±1.6 (5)	11.9±3.6 (6)
	BC_{tot}	24.0±6.1 (5)	27.7±5.6 (6)	8.7±4.0 (5)	22.2±9.0 (6)
	TSB (s)	2784±678.7 (5)	1430±347.6 (6)	768±436.1 (5)	1210±522.5 (6)
	$TSB_{anaerob}$ (%)	1.7±1.0 (5)	2.3±0.4 (6)	1.2±0.6 (5)	1.3±0.4 (6)
1170 µatm CO_2	BC_{max}	7.6±1.6 (5)	11.8±2.9 (6)	6.2±1.5 (5)	4.0±1.2 (4)
	BC_{tot}	18.1±3.4 (5)	22.7±6.6 (6)	12.5±4.7 (5)	6.5±1.9 (4)
	TSB (s)	2280±251.0 (5)	1610±343.4 (6)	1104±481.7 (5)	945±279.7 (4)
	$TSB_{anaerob}$ (%)	0.8±0.1 (5)	1.4±0.4 (6)	1.1±0.3 (5)	0.9±0.3 (4)

All values are means±s.e.m. Number of individuals is shown in brackets. BC_{max}, maximum burst count per 30 s; $TSB_{anaerob}$, putative proportion of anaerobic metabolism during the period between U_{gait} and U_{crit} with an estimated duration of 1 s per burst.

15% of the resting $\dot{M}_{O_2}$ values to fall below the actual individual SMR (Dupont-Prinet et al., 2013; Chabot et al., 2016).

The SMRs of *B. saida* acclimated long-term at 3 and 6°C were similar to those found in the 0°C acclimated individuals. This implies efficient metabolic compensation in the thermal range between 0 and 6°C (Precht, 1958). Metabolic compensation is hypothesized to enable the individual to maintain vital functions independent of environmental temperatures (Precht, 1958). Hop and Graham (1995) detected incomplete compensation in the SMR of *B. saida* following a 12 day exposure to 2.7°C compared with SMR values obtained in specimens acclimated for 5 months at 0.4°C. Accordingly, the rather short exposure period (12 days) to the elevated temperature was probably insufficient to establish a new physiological steady state and thereby to unfold the full acclimation potential of this species. At 8°C, the SMR of *B. saida* was significantly elevated, even after 4 months of acclimation, also perceived in a non-significantly reduced growth performance (Kunz et al., 2016a). Mitochondrial plasticity has been identified to be involved in setting the limits of thermal acclimation capacity (e.g. Strobel et al., 2013). Accordingly, the elevated whole-animal SMR at the highest temperature investigated may, at least partly, be attributed to limited acclimation capacities expressed through reduced mitochondrial efficiencies at 8°C shown in cardiac myocytes of the same individuals as used in the present study (Leo et al., 2017). *B. saida* revealed little capacity to adjust mitochondrial enzyme activities and lipid class compositions in response to warm acclimation above 6°C (Elettra Leo, Martin Graeve, Daniela Storch, H.-O.P. and F.C.M., unpublished data). Accordingly, an enhanced proton leak and an associated decrease in ATP production efficiency evoked by changes in membrane fluidity are suggested to cause the impaired mitochondrial efficiency in *B. saida* close to its long-term whole-animal upper thermal tolerance limit [pejus temperatures (T_{pej}) *sensu* Pörtner et al., 2017; Leo et al., 2017].

Elevated CO_2 levels did not influence the SMR of *B. saida* in the present study in line with findings for the Atlantic cod (*Gadus morhua*) (3–4 weeks of exposure, Kreiss et al., 2015) and the Antarctic *Notothenia rossii* (29–36 days of exposure, Strobel et al., 2012), long-term acclimated to moderate P_{CO_2} conditions. This suggests a rather low sensitivity of baseline metabolism to hypercapnia. Accordingly, the resting cardiac mitochondrial respiration of *B. saida* was not affected by chronically elevated P_{CO_2} (Leo et al., 2017).

Recorded values for MMR are consistent with recently published results for *B. saida* (Drost et al., 2016). A positive correlation between MMR and environmental temperatures as detected in the present study is well established for diverse teleost species (e.g. Claireaux et al., 2006; Eliason et al., 2011; Clark et al., 2011) along with an increase in cardiorespiratory performance with temperature. Limitations in heart rate and oxygen-carrying capacity are hypothesized to cause a levelling off in MMR at high acclimation temperatures (Pörtner, 2010), as seen in the present study.

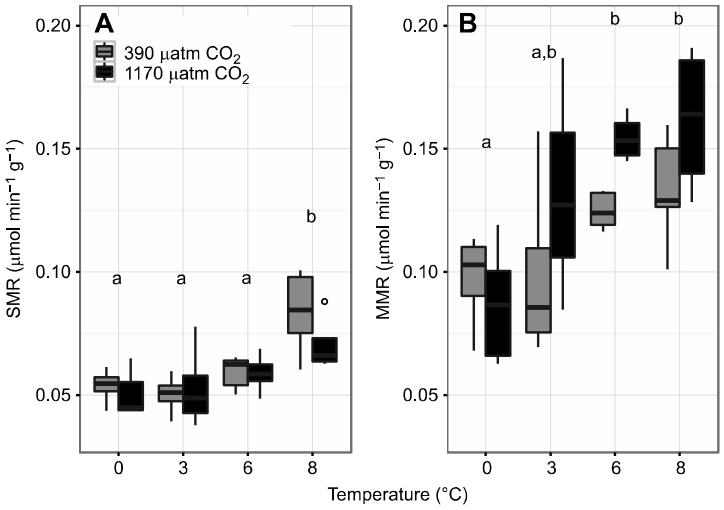

Fig. 1. Standard metabolic rate (SMR) and maximum metabolic rate (MMR) in polar cod (*Boreogadus saida*) over a range of acclimation temperatures at two P_{CO_2} levels. Boxplots (mean±s.e.m.) of (A) SMR and (B) MMR at four different temperatures and two P_{CO_2} levels, as indicated. Full data are summarized in Table 3. Letters indicate results of Tukey honest significance test between temperature treatments (SMR: P<0.0001; MMR: P=0.0005). Significant differences are represented by different letters. A significant P_{CO_2} effect was detected only in MMR (SMR: P=0.343; MMR: P=0.0322); no interaction effect (two-way ANOVA) was observed (SMR: P=0.470; MMR: P=0.2577) (see Table 1). All boxes show 25th and 75th percentiles with median; whiskers are 5th and 95th percentiles.

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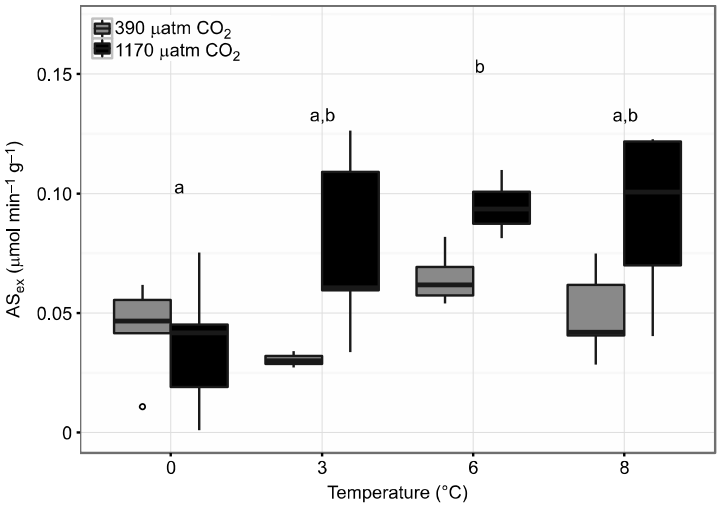

Fig. 2. Aerobic scope (AS_{ex}) for exercise in *B. saida* over a range of temperatures at two P_{CO_2} levels. Boxplots (mean±s.e.m) of AS_{ex} at four different temperatures and two P_{CO_2} levels, as indicated. Full data are summarized in Table 3. Letters indicate results of Tukey honest significance test between temperature treatments (P=0.0185). Significant differences are represented by different letters. A significant P_{CO_2} effect was detected (P=0.0059); no interaction effect (two-way ANOVA) was observed (P=0.1233) (see Table 1).

The continuous increase in MMR with temperature and the elevated SMR at 8°C result in a peak aerobic scope for exercise (AS_{ex}) of *B. saida* acclimated to 6°C. In line with this observation, growth of *B. saida* under laboratory conditions was also maximum at 6°C (Kunz et al., 2016a), suggesting a connection between aerobic capacities and growth as well as exercise (Pörtner and Knust, 2007; Pörtner and Farrell, 2008). The optimization of aerobic performance governed by environmental factors is widely recognized to determine a species' spatial and temporal niche (Claireaux and Lagardère, 1999; Pörtner and Farrell, 2008). Nevertheless, fish often inhabit areas with temperatures well below their physiological optimum obtained under artificial *ad libitum* food situations (Björnsson et al., 2001) indicating that maximum exploitation of aerobic scope is not a precondition for survival (Deutsch et al., 2015; Norin and Clark, 2016). Despite cold-induced reductions in AS_{ex} due to lower MMR, *B. saida* is well adapted to temperatures around 0°C, visible in a high feed conversion efficiency (Kunz et al., 2016a). Likewise, Hop et al. (1997) found assimilation efficiencies at 0°C (average 80%), similar to assimilation capacities detected in stenothermal Antarctic fish. Thus, *B. saida* likely has an energetic advantage over potential predators and competitors in cold waters. In contrast, when inhabiting waters with their maximum AS_{ex} and their optimum temperature for growth (6°C) (Kunz et al., 2016a), a reduced abundance of suitable prey (Fossheim et al., 2015) would probably demand elevated energy fractions for foraging activity, possibly constraining growth and reproduction. Accordingly, during ongoing climate change, polar fish at their southern distribution limits such as *B. saida* may be especially vulnerable to competition with invading species adapted to higher water temperatures. Among the northwards moving species, capelin (*Mallotus villosus*), Atlantic cod and haddock (*Melanogrammus aeglefinus*) represent a potential treat to *B. saida*. While *M. villosus* is expected to compete for prey with *B. saida* during a progressive future habitat overlap (Hop and Gjøsæter, 2013), juvenile *G. morhua* and *M. aeglefinus* revealed little dietary overlap with *B. saida* in habitats where they

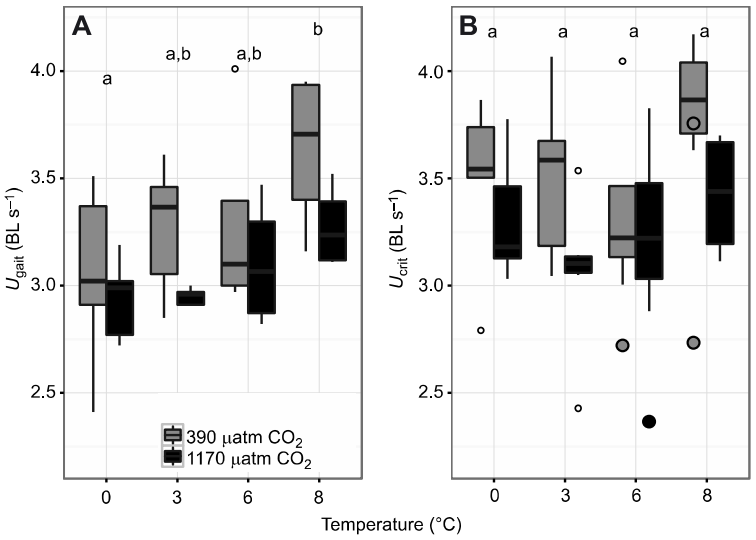

Fig. 3. Gait transition speed (U_{gait}) and critical swimming speed (U_{crit}) in *B. saida* over a range of acclimation temperatures at two P_{CO_2} levels. Boxplots (mean±s.e.m.) of (A) U_{gait} and (B) U_{crit} (adjusted according to Brett, 1964) at four different temperatures and two P_{CO_2} levels, as indicated. Filled circles are U_{crit} of individuals without burst capacity (not included in statistical analysis). Full data are summarized in Table 3. Letters indicate results of Tukey honest significance test between temperature treatments (U_{gait}: P=0.0341; U_{crit}: P=0.2014). Significant differences are represented by different letters. A significant P_{CO_2} effect was solely detected in U_{gait} (U_{gait}: P=0.0270; U_{crit}: P=0.0559); no interaction effect (two-way ANOVA) was observed (U_{gait}: P=0.8134; U_{crit}: P=0.8299) (see Table 1).

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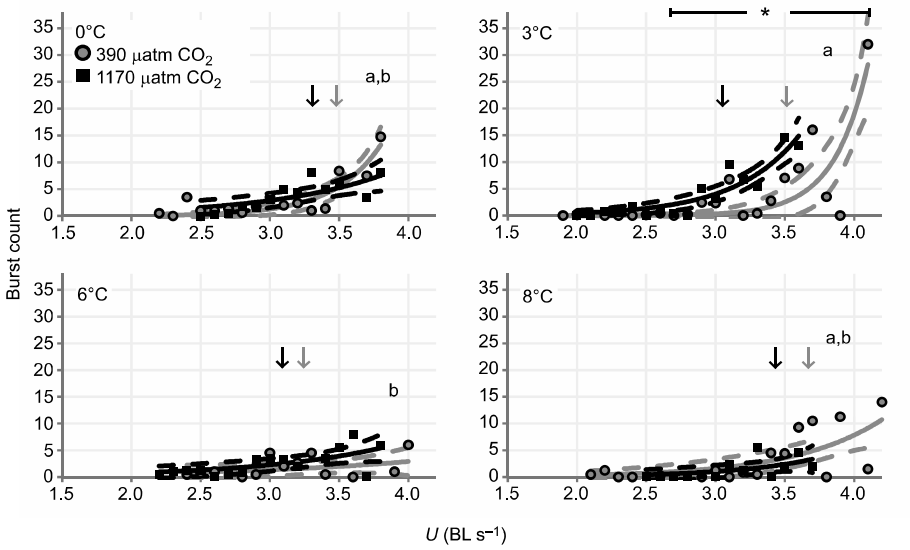

Fig. 4. Mean number of bursts per treatment per velocity step (BL s^{-1}) in *B. saida* over a range of acclimation temperatures at two P_{CO_2} levels. Arrows indicate mean U_{crit} per treatment (note that U_{crit} values of individuals without burst capacity were included in the calculation of mean U_{crit}). Solid lines represent a data fit to a nonlinear regression [mean burst count (U) = $a \times exp(U \times b)$]. Dashed lines are 95% confidence intervals (CI) of nonlinear regression. Asterisk indicates significant P_{CO_2} effect (non-overlapping 95% CI). Letters show results of Tukey honest significance test for maximum burst count (BC_{max}) between temperature treatments (P=0.0075). Significant differences are represented by different letters. A significant P_{CO_2} effect was detected for BC_{max} (P=0.0231); no interaction effect (two-way ANOVA) was observed for BC_{max} (P=0.3623) (see Table 1).

co-occurred (Renaud et al., 2012). During ongoing climate change, however, adult *G. morhua* and *M. aeglefinus* may become increasingly important as predators on *B. saida* (Renaud et al., 2012). Nevertheless, the distribution of *B. saida* has already been observed to contract in its southern habitat as a direct result of increasing water temperatures (Eriksen et al., 2015).

In parallel to AS_{ex}, both mainly aerobically fuelled steady-state swimming performance (Lurman et al., 2007) (U_{gait}) and partly anaerobically fuelled U_{crit} are known to increase acutely with temperature up to maximum performance before decreasing at temperatures approaching the critical thermal limit ($T_{C,max}$ *sensu* Farrell, 2016) (e.g. Griffiths and Alderdice, 1972). In the present study, however, U_{gait} of *B. saida* showed only a modest increase with acclimation temperature. U_{crit} did not reveal any clear trend with long-term acclimation temperature. These results indicate that metabolic compensation processes during warm acclimation as observed for the SMR of *B. saida* (see above) were also reflected in swimming metabolism for this species. Thus, while U_{gait} and U_{crit}

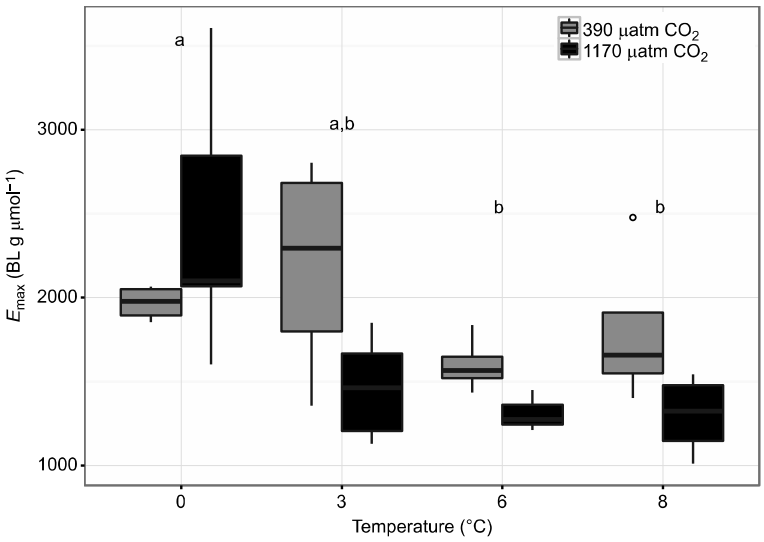

Fig. 5. Efficiency of maximum swimming performance (E_{max}) in *B. saida* over a range of acclimation temperatures at two P_{CO_2} levels. Boxplots (means±s.e.m.) of E_{max} at four different temperatures and two P_{CO_2} levels, as indicated. Full data are summarized in Table 3. Letters indicate results of Tukey honest significance test between temperature treatments (P=0.0085). Significant differences are represented by different letters. No significant difference between P_{CO_2} treatments was found (P=0.1045). An interaction effect (two-way ANOVA) was observed (P=0.0465) (see Table 1).

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of *B. saida* showed signs of acclimation throughout the range of investigated temperatures (0–8°C), the SMR of *B. saida* showed full compensation up to only 6°C attributed to a significant reduction in mitochondrial ATP production efficiency at 8°C compared with lower acclimation temperatures (0, 3 and 6°C) (Leo et al., 2017). The decreasing mitochondrial ATP production efficiency may further contribute to the observed decrease in muscle output efficiency (here expressed as E_{max}) with acclimation temperature. Based on the reduced mitochondrial efficiencies that translated into organismic limitations, we expect an overall limited capacity of *B. saida* to acclimate to water conditions higher than 6°C. Similar to indications obtained in our study, Drost et al. (2016), who investigated the thermal acclimation response of *B. saida* from the Canadian Arctic by measuring cardio-respiratory performance, found that *B. saida* can acclimate to 6.5°C (highest investigated acclimation temperature). Nevertheless, cardio-respiratory limitations caused a higher sensitivity of 6.5°C-acclimated specimens to acute temperature changes compared with *B. saida* acclimated to lower temperatures (Drost et al., 2016).

The switch from aerobic to anaerobic metabolism at U_{gait} is marked by burst-type exercise events (Milligan and Wood, 1986; Lurman et al., 2007). Burst performance is essential during predator–prey interactions (Beamish, 1978), and can only be maintained for short periods. *B. saida* showed low burst capacity throughout all temperature/P_{CO_2} treatments (Table 4), with a few specimens (n=4 at 6 and 8°C treatments out of in total 42 specimens used in the swimming performance tests) not even displaying any burst behaviour at all. Accordingly, the contribution of anaerobic metabolism to maximum swimming capacity is putatively minor. This phenomenon is in line with observations in other polar species, including Antarctic fishes that revealed low potential for anaerobic glycolysis (e.g. the yellowbelly rockcod *Notothenia neglecta*, Dunn and Johnston, 1986; the bald notothen *Pagothenia borchgrevinki*, Davison et al., 1988). Compared with active temperate species, the burst performance of *B. saida* is several-fold lower (highest maximum burst count for *B. saida*: 19.5 for 30 s, 3°C versus e.g. European sea bass *Dicentrarchus labrax*, 23°C; maximum burst count at U_{crit}: ~84 for 30 s, Marras et al., 2010). Furthermore, the stores of the white muscle metabolites ATP and glycogen have been shown to remain independent of acclimation temperature (see review by Kieffer, 2000). Combined with the low level of anaerobically fuelled swimming capacity (0–5.7%), the slightly elevated burst performance (both BC_{max} and BC_{tot}) of *B. saida* at 3°C found in the present study is not given much weight. Hence, the moderately active lifestyle of *B. saida* described by Gradinger and Bluhm (2004) is also mirrored in the low-burst swimming performance, possibly involving a disadvantage during predator avoidance.

The response of aerobic capacities to near-future elevated P_{CO_2} conditions has been found to be strongly species specific (compare review by Esbaugh, 2018), with reduced sensitivities to CO_2 suggested for species frequently exposed to natural fluctuations in abiotic conditions (Rummer et al., 2013). The majority of studies did not detect any impact of near-future P_{CO_2} conditions on MMR in a variety of species after chronic exposure (*G. morhua*, 4 months at 3000 µatm, Melzner et al., 2009; red drum, *Sciaenops ocellatus*, 14 days at 1000 µatm, Esbaugh et al., 2016; blue rockfish, *Sebastes mystinus* 16–19 weeks at 750, 1900 and 2800 µatm, Hamilton et al., 2017). In contrast, a reduction of MMR under ocean acidification was found in copper rockfish (*Sebastes caurinus*) exposed to 1900 µatm CO_2 and 10°C for 14–17 weeks (Hamilton et al., 2017). *B. saida*, however, revealed an elevated MMR under near-future P_{CO_2} conditions (at all temperatures above 0°C), in line with observations in the tropical coral reef fish *Acanthochromis polyacanthus* (17 days at 950 µatm, Rummer et al., 2013) and the temperate species *D. labrax* under long-term exposure to realistic P_{CO_2} scenarios (1.5 years, 1500 µatm, Amélie Crespel, Katja Anttila, Pernelle Lelièvre, Patrick Quazuguel, Nicolas Le Bayon, José-Luis Zambonino-Infante, Denis Chabot and G.C., unpublished data). Interestingly, *B. saida* showed reduced maximum swimming velocities despite elevated MMR. Unfortunately, the studies of Rummer et al. (2013) and Crespel and coworkers (unpublished data) do not report on swimming performance. As a consequence of elevated MMR and reduced swimming performance, E_{max} was impaired in *B. saida* under hypercapnia at all temperatures above 0°C. Under control P_{CO_2} conditions, however, swimming performance efficiency was only reduced at acclimation temperatures above 3°C, suggesting a higher thermal sensitivity of MMR under hypercapnia. Nevertheless, E_{max} has to be considered with care as an unquantifiable oxygen debt may interfere with it (Brett, 1962), which, in turn, is expected to be of little extent due to limited anaerobic capacities implied by a low burst capacity detected in the present study.

To date, physiological mechanisms causing elevated aerobic metabolic costs under moderate P_{CO_2} conditions are not fully understood. Our results suggest that this cost is likely elevated through mechanisms other than exercise and constrains swimming performance (both U_{gait} and U_{crit}) to lower levels under hypercapnia while causing elevated $\dot{M}_{O_2}$ and a consequently higher AS_{ex}. One organ potentially being involved in elevated energy demands under high P_{CO_2} conditions is the gill. Kreiss et al. (2015) found branchial $\dot{M}_{O_2}$ per gram gill tissue after long-term acclimation of *G. morhua* to 2200 µatm remained comparable to values under control P_{CO_2} conditions at 10°C. However, high P_{CO_2} caused an increase in gill soft tissue, resulting in elevated fractions of gill $\dot{M}_{O_2}$ from whole-animal $\dot{M}_{O_2}$ (increase from 5 to 7%) (Kreiss et al., 2015). Nevertheless, the SMR of both *G. morhua* (Kreiss et al., 2015) and *B. saida* (present study) remained unaffected under hypercapnia. Yet, potential cascading effects might be amplified during maximum performance causing trade-offs in swimming capacity as seen in *B. saida*. The results of the present study focusing on whole-animal parameters, however, represent only an ensemble of organismic costs and do not give further insight into the energetic partition of underlying mechanisms.

Hypercapnia-acclimated fish not only showed a shift to anaerobic white muscle reserves at lower swimming speeds (as observed by lower U_{gait}), but the burst performance was also reduced compared with normocapnia-acclimated fish. Despite a marginal contribution of anaerobic metabolism to the maximum swimming capacities of *B. saida*, this finding is in line with the overall impairment in maximum performance detected following exposure to elevated P_{CO_2} levels. Hence, aerobic as well as anaerobic exercise capacities appear reduced under high P_{CO_2} scenarios. Thus, hypercapnia has effects on the energy metabolism of *B. saida* at high and maximum metabolic rates that are not visible at rest (Kunz et al., 2016a).

In conclusion, the present study revealed a strong impact of ocean acidification on maximum performance traits of *B. saida*. Although elevated P_{CO_2} levels did not significantly impact routine parameters (growth, food consumption, SMR) in this species (Kunz et al., 2016a), trade-offs in energy allocation became visible when the metabolism was operating at maximum performance under hypercapnic conditions. More precisely, long-term acclimation under near-future P_{CO_2} conditions caused reduced swimming capacity of *B. saida* at higher metabolic costs. Consequently, when translating the present results obtained from a limited number

of specimens onto the population level, foraging success and escape response of *B. saida* during predator encounters might be impaired under future water conditions. Species that are resilient to a broader range of abiotic conditions, such as *G. morhua* (Melzner et al., 2009), may find it easier to prevail in light of the ongoing borealization and community shifts in the Arctic (Fossheim et al., 2015). Accordingly, the competitive strength of *B. saida*, and thereby its abundance in this new setting in the waters around Svalbard can be expected to decrease.

Acknowledgements
We gratefully acknowledge J. Nahrgang for providing *B. saida* (research program Polarisation no. 214184/F20 funded by the Norwegian Research Council), as well as the crew of RV Helmer Hanssen (University of Tromsø) for animal collection. We would like to thank E. Leo, M. Schmidt, S. Hardenberg and H. Windisch for their support during the realization of the incubation setup and animal maintenance, as well as T. Hirse and S. Berger for technical assistance with the manipulation of CO_2 partial pressure. We appreciate the contribution of A. Tillmann, I. Ketelsen, F. V. Moraleda, K. Zanaty, M. Machnik and B. Matthei to the measurements of pH and DIC. Finally, we greatly appreciate the constructive suggestions of the two reviewers.

Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: K.L.K., H.P., R.K., F.C.M.; Methodology: K.L.K., G.C., F.C.M.; Validation: K.L.K., G.C.; Formal analysis: K.L.K.; Investigation: K.L.K., F.C.M., G.C.; Data curation: K.L.K.; Writing - original draft: K.L.K., F.C.M.; Writing - review & editing: K.L.K., G.C., H.P., R.K., F.C.M.; Supervision: G.C., H.P., R.K., F.C.M.; Project administration: F.C.M.; Funding acquisition: H.P., R.K., F.C.M.

Funding
This project was part of the research program BIOACID (Biological Impacts of Ocean Acidification, phase II) funded by the German Bundesministerium für Bildung und Forschung (BMBF; FKZ 03F0655B and FKZ 03F0728B to H.-O.P., R.K. and F.C.M.). K.L.K., H.-O.P., R.K. and F.C.M. acknowledge funding through the PACES (Polar Regions and Coasts in a Changing Earth System) program of the Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI). Deposited in PMC for immediate release.

Data availability
Raw data generated and analyzed during the present study are available in PANGAEA under deposition number 889447 (Kunz et al., 2018).

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PUBLICATION III

Influence of water temperature and feeding status on aerobic metabolic scope and swimming performance of Polar cod (*Boreogadus saida*)

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submitted: 6 May 2018

Influence of water temperature and feeding status on aerobic metabolic scope and swimming performance of Polar cod (*Boreogadus saida*)

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Key words: Arctic cod, SDA, metabolic prioritization, climate change, critical swimming speed, maximum metabolic rate, food deprivation

Abstract

Periods of extensive aerobic exercise coinciding with digestion require metabolic prioritizations in most fish species. When seawater temperatures are rising, prioritizations that have evolved depending on the species' life-style may no longer be in line with metabolic balance causing species-specific changes in aerobic capacities. Pronounced increases in temperature are projected for Arctic regions. Therefore, we acclimated Polar cod (*Boreogadus saida*) to current ambient (0°C) as well as projected (6°C) temperatures and investigated their swimming and digestive performance. Further, we assessed whether different acclimation histories affected metabolic performance during consecutive short-term exposure to 3°C. Food-deprived and fed *B. saida* were exercised at incremental swimming speeds after acclimation to 0 and 6°C (71 and 45 days, respectively) in order to determine maximum aerobic swimming velocities and associated metabolic rates. At both acclimation temperatures, the aerobic scope for digestion (post-prandial increase in oxygen consumption) was lower than the aerobic scope for exercise. Our results further indicate limited aerobic capacities of *B. saida* at 0°C resulting in exercise constraints in fed animals. At 6°C, aerobic capacities rose, allowing exercise and digestion to take place simultaneously in fed individuals, while energy shortage likely limited the swimming performance unfed fish. Consecutive exposure to 3°C revealed equal swimming performances in both acclimation groups, suggesting limited warm-acclimation capacities. In conclusion, food deprivation prohibits *B. saida* from exploiting the full potential of aerobic swimming capacities at 6°C which indicates a competitive disadvantage for this species in future oceans characterized by reduced prey availability.

Introduction

Locomotion is an energy-demanding process, causing an increase in cardio-respiratory activity and enhanced blood flow to the active skeletal muscle due to elevated O_2 demand (Hicks and Bennett 2004). Locomotion of ectotherm organisms integrates biochemical, morphological and physiological traits (Arnold 1983). Therefore, swimming performance is tightly linked to the fitness of fish, determining their individual success during predator-prey interactions, escape and migratory movements at ecosystem level (Baker 1978; Webb 1986; Reidy et al. 1995). An individual's exercise capacity is commonly assessed during incremental swimming tests in a fasted state involving the determination of the critical swimming speed (U_{crit}) (Plaut 2001; MacNutt et al. 2004). These tests are usually paralleled by simultaneous recordings of oxygen consumption ($\dot{M}O_2$) for the evaluation of the respective metabolic expenditure for swimming. The maximum metabolic rate obtained at U_{crit} (MMR_{ex}) is widely considered to represent an individual's maximum aerobic metabolic capacity (Muir and Niimi 1972).

However, exercise is only one process drawing from the so-called aerobic power budget (Guderley and Pörtner 2010). Another major player is digestion, which causes a post-prandial increase in $\dot{M}O_2$ termed specific dynamic action (SDA). The elevated metabolic rate due to digestion is evoked by upregulated processes (Secor and Diamond 1998) necessary for the assimilation and transformation of nutrients (e.g. Jobling 1981; Brown and Cameron 1991a, b; Houlihan 1991), with the major energetic fraction attributed to the costs for protein synthesis (Brown and Cameron 1991a, b; Jobling 1993). Hence, the biochemical processes during digestion are essential for energy provision and growth (Jobling 1981; Jourdan-Pineau et al. 2009). The magnitude and duration of SDA depends on meal size (e.g. Beamish 1974; Brett 1976; Jobling and Spencer Davies 1980; Jordan and Steffensen 2007) and food composition (Jobling and Spencer Davies 1980; Jobling 1981). Depending on species and lifestyle, the aerobic scope for digestion can approach or even exceed the increment in $\dot{M}O_2$ associated with exercise (Hicks and Bennett 2004).

In the field, locomotion and digestion often need to be accomplished simultaneously (e.g. Hicks and Bennett 2004; Thorarensen and Farrell 2006), especially at low water temperatures that cause extended SDA durations (Hop and Graham 1995). Species characterized by extensive aerobic capacities may additively process these combined metabolic needs, an ability considered to be beneficial for survival (Thorarensen and Farrell

2006). This additive metabolic pattern, which has been observed in various fish species (Dupont-Prinet et al. 2009; Jourdan-Pineau et al. 2009; Fu et al. 2009; Li et al. 2010), becomes visible in the capacity to increase the maximum metabolic rate further when both exercise and digestion (MMR_{ex+dig}) need to be maintained. In other species, however, constraints in the transport capacity of the cardiopulmonary system are causing prioritizations during combined aerobic processes according to species-specific physiological strategies: Fed rainbow trout (*Oncorhynchus mykiss*) and chinook salmon (*Oncorhynchus tshawytscha*) did not show the capacity to enhance their MMR_{ex+dig} above the MMR_{ex} of unfed specimens. Consequently, fed fish revealed a reduced U_{crit} (Alsop and Wood 1997; Thorarensen and Farrell 2006), emphasizing a role for different species-specific lifestyles (in terms of behavior and foraging strategies) (Clark et al. 2013). Active piscivore species generally possess high swimming capacities affected marginally by digestion. Rather inactive ambush predators, in turn, are characterized by high capacities to accommodate digestive processes and low metabolic investments in aerobic swimming performance (Clark et al. 2013).

Both swimming metabolism and SDA response are highly influenced by varying abiotic conditions (e.g. temperature: Claireaux et al. 2006; Jobling and Spencer Davies 1980; hypoxia: Petersen and Gamperl 2010; Jordan and Steffensen 2007; hypercapnia: Hamilton et al. 2017; Kunz et al. 2018b). Due to the temperature-dependency of physiological processes, temperature is considered as the most powerful abiotic factor controlling the metabolic performance of ectotherms (Fry 1947). Yet, studies investigating the partitioning of aerobic scope among competing metabolic processes in fish focused predominantly on hypoxia (Dupont-Prinet et al. 2009; Jourdan-Pineau et al. 2009; Zhang et al. 2010) as abiotic stressor, due to its constraining effect on aerobic metabolic performance (Chabot and Claireaux 2008). Only very few studies have considered temperature-effects on species-specific metabolic strategies (e.g. Pang et al. 2011).

However, the role of temperature is becoming more and more prominent. Sea surface temperature is changing in the Arctic at a rapid pace due to ongoing climate change (Pörtner et al. 2014). According to the business-as-usual CO_2 emission scenario (RCP 8.5), a rise in sea surface temperature of up to 5 - 11°C is projected for the Arctic by the end of the century (IPCC 2014). The present study, therefore, focusses on an Arctic key species the Polar cod (*Boreogadus saida*). Due to its high energy content (Harter et al. 2013) and high abundance (Mueter et al. 2016 and references therein), *B. saida* plays an important role in the Arctic

food web as a prey organism for various taxa (e.g. Bradstreet et al. 1986). The cold-adapted stenothermal *B. saida* preferably inhabits niches with subzero temperatures and is rarely found above 3.2°C (Falk-Petersen et al. 1986). As juveniles, however, *B. saida* are found in waters up to 5.2°C (Falk-Petersen et al. 1986). *B. saida* is characterized as moderately active (Gradinger and Bluhm 2004) with a low growth potential (Laurel et al. 2015; Kunz et al. 2016). Although the ecological foundation for aerobic swimming capacity of *B. saida* is not fully resolved, current studies imply potential under-ice migrations of this species (David et al. 2015). Consequently, a potential decrease in aerobic swimming performance of *B. saida* in response to rapidly changing abiotic conditions might entail a decline in its abundance with an unpredictable impact on the Arctic ecosystem.

The aim of the present study was the investigation of potential conflicts between the O_2 demands of locomotion and digestion in *B. saida*. Shifts in energy allocation of *B. saida* were investigated depending on seawater temperatures with the goal to evaluate this species' future fitness under realistic climate change scenarios. Finally, the impact of acclimation history on the metabolic performance capacity of *B. saida* was investigated during short-term exposure to a common intermediate temperature in order to identify potential differences.

Material and Methods

Animal collection

Boreogadus saida (Lepechin, 1774) were caught in September 2015 by bottom trawl on expedition HE451.1 of RV *Heincke* in the cold (-1.7 – 0°C) bottom layers in Kongsfjorden (78° 58.92' N 11° 45.99' E) and Billefjorden (78° 36.37' N 16° 30.74' E), Svalbard (for further information about the cruise, see Mark (2015)). To protect the fish from physical stress, a fish-lift (Holst and McDonald 2000) was connected to the cod end of the bottom trawl. On board, the fish were kept in a thermostatted recirculating aquarium system (4 m^3) until arrival to the laboratories of the Alfred Wegener Institute (AWI) in Bremerhaven, Germany. Prior to acclimation to the experimental conditions, all specimens were kept at 1.5°C for approx. two weeks.

Experimental design

Two groups of *B. saida* ($n = 8$, $n = 5$) were incubated at both 0°C (8.9 – 19.1 g, mean: 11.6 g ± 0.9; 10.6 – 14.1 cm, mean: 11.9 cm ± 0.3) and 6°C (5.4 – 14.2 g, mean: 10.7 g ± 0.7; 9.8 – 13.0 cm, mean: 11.7 cm ± 0.3) for in total 125 and 102 days, respectively. The temperatures were chosen, because 0°C represents the habitat temperature of *B. saida*, while 6°C represents an elevated water temperature according to RCP 8.5, which has also been identified as the optimum temperature of aerobic scope under laboratory conditions (Kunz et al. 2018b). The two groups of *B. saida* were kept in separated aquaria of approx. 45 L each. In order to guarantee identical water conditions for both groups, the aquaria were connected to a common pre-conditioned (0 and 6°C, respectively) water body (~ 1000 L). The experimental temperatures throughout the present study were maintained by aid of thermostatted rooms. Water temperature (WTW LF 197 multimeter, WTW, Weilheim, Germany), as well as NH_4^+ levels (photometric test kits, Macherey-Nagel, Düren, Germany) were measured twice a week. A NH_4^+ value of 0.4 mg L^{-1} was set as threshold for partial water exchanges (approx. 600 L). During the first six weeks of incubations, the fish were fed daily with baby krill (*Euphausia pacifica*) (Erdman, Ritterhude, Germany) in the common tank. Consecutively, feeding was reduced to three times a week. Four days prior to SDA or exercise measurements of fed fish, feeding occurred daily to ensure full satiation. We decided not to perform force-feeding and let the fish feed voluntarily in

groups in order to stimulate food intake across individuals as suggested by Hop and Tonn (1998). This prevented artificial increases in $\dot{M}O_2$ due to handling stress and reflected natural conditions best. The amount of consumed food was expressed as daily ration. Each ration was patted dry with paper towels before weighing for the determination of meal size. Lights were dimmed except during feeding events, measurements of water condition and swim tunnel experiments.

SDA

After an acclimation time of at least two weeks at the respective temperature, the specific dynamic action (SDA) of *B. saida* (n = 5) was recorded for 18 days at 0°C and for 9 days at 6°C by automated intermittent-flow respirometry. The relatively long measurement periods were established due to rather slow gastric evacuation rates reported for *B. saida* at ambient temperature (evacuation half-time: 146 h, -0.49°C) (Hop and Tonn 1998). During SDA measurements, the specimens had a total length of 11.4 – 12.8 cm (mean: 11.8 cm ± 0.3) (0°C) and 9.8 – 12.1 cm (10.9 cm ± 0.4) (6°C) and a wet weight of 9.7 – 14.8 g (mean: 11.7 g ± 0.9) (0°C) and 5.4 – 11.2 g (mean: 8.5 g ± 1.1) (6°C). Lengths and weights of individual *B. saida* were considered prior to SDA measurement (full gastrointestinal system) and after SDA termination (empty gastrointestinal system). Four days prior to the experiment, the feeding frequency was increased to a daily schedule. The *ad libitum* food uptake was 3.4% BW and 4.4% BW for 0 and 6°C, respectively. After a feeding period of two hours in the common aquarium, the fish were transferred into an intermittent flow respirometry setup, comprising acryl respiration chambers (1.815 L or 2.210 L) that were submerged in separate tanks (~ 45 L). In order to guarantee O_2 saturation, the water body surrounding each respiration chamber was constantly aerated with compressed air. Water temperature was continuously monitored with a WTW LF 97 multimeter. Furthermore, water quality was maintained during the respiration measurements by daily partial water exchanges and periodic verifications of the NH_4^+ concentration. An opaque lid on each aquarium prevented measurements from being influenced by potential disturbances.

For 0%-calibration, the optical oxygen probes (Fibox, PreSens Precision Sensing GmbH, Regensburg, Germany) were flushed once with water-saturated N_2 at room temperature, while the 100%-calibration was done prior to each measurement round in O_2-saturated water at the respective experimental temperature. Periods of $\dot{M}O_2$ measurement were

interrupted by automated flushing periods (0°C, 60:30 min; 6°C, 45:45 min) in order to reestablish O_2 saturation within the respiration chambers. The flush period was initiated by an aquarium pump (300 L h^{-1}). A circulation pump (490 L h^{-1}) created a constant water flow over the oxygen probe. The O_2 concentration was continuously recorded by five Fibox 3 (6°C) or by a ten-channel oxygen meter (0°C) (both PreSens Precision Sensing GmbH, Regensburg, Germany). The resulting slope of O_2 concentrations between two flush periods was used to calculate $\dot{M}O_2$. Blank measurements of bacterial background respiration were carried out in one spare respiration chamber in parallel with the SDA experiment (0°C) or were recorded for several hours subsequent to the SDA measurement (6°C).

Swimming performance

The fish were acclimated to 0°C (n = 7) and 6°C (n = 8) for 71 and 45 days, respectively, prior to performance capacity experiments in a swim tunnel (30 L, dimension working section: 46.5 x 13.5 x 14 cm, Loligo Systems ApS, Denmark). During the first round of swimming performance measurements, the fish had a total length of 11.6 – 14.6 cm (mean: 12.9 cm ± 0.4) (0°C) and 11.8 – 13.5 cm (mean: 12.5 cm ± 0.2) (6°C) with a wet weight of 10.0 – 20.1 g (mean: 13.8 g ± 1.5) (0°C) and 10.8 – 16.5 g (mean: 13.4 g ± 0.8) (6°C) (compare Table 1). An identical swimming protocol (see below) was applied three times for both groups, once with fed specimens, once with starved specimens (starvation period: 18 – 21 days, 0°C; 10 – 13 days, 6°C) and once with fed individuals (acclimated to 0°C and 6°C, respectively) acutely exposed to 3°C. The chosen protocol allowed the completion of two swimming trials per day.

A laminar flow within the working section of the swim tunnel was maintained by aid of a honeycomb-shaped plastic device. A second honeycomb device at ~ 13 cm distance from the first one (new length of working section: 33.5 cm) narrowed the width of the swim tunnel corner with decreased water velocity and prevented the possibility to rest during the swimming protocol. Calibration of water velocity to voltage output from the control unit was accomplished with a flow sensor (Vane wheel flow sensor FA, Höntzsch Instruments, Waiblingen, Germany) inserted in the center of the working section. An opaque plastic curtain was installed to cover the swim tunnel in order to minimize potential disturbances during the experiment.

In case of experiments with fed fish, the specimens were allowed to feed for precisely two hours in the common tank with a predetermined amount of food, before the first fish was transferred into the swimming section of the tunnel. The food remained in the common tank until the first swimming trial was finished and the second fish was placed in the swim tunnel. Immediately afterwards (approx. six hours after food exposure), the remaining food items were removed and weighed. In further experiments exposing the fish acutely to 3°C, the specimens were allowed to feed for two hours at their respective acclimation temperature, before they were transferred into the swim tunnel, which held a water temperature of 3°C. The subsequent experimental procedure was identical to the one applied at acclimation temperature (see below).

Prior to the start of the exercise protocol, the fish were allowed to acclimate to the swim tunnel and to recover from handling stress without water current for exactly two hours. Therefore, the swimming protocol for fed fish started 4h or 8h after feeding, which corresponds to the time frame for maximum metabolic rate of digestion (MMR_{dig}) in resting fish. After the acclimation period, the protocol also involved a settling period of 5 min to a baseline swimming speed (26 cm sec^{-1}), before the speed was increased once per minute in steps of 1.4 cm sec^{-1}, thereby being closely related to a constant acceleration test (CAT). Throughout the period of the exercise protocol, burst swimming events were counted (BC) at every velocity step for approx. 50 sec. Thereby, the gait transition (U_{gait}) of each individual was determined, which characterizes the transition between aerobic sustained and anaerobic metabolism. The protocol ended as soon as the critical swimming speed (U_{crit}) was reached, defined as the velocity at which the fish touched the grid for at least 30 sec.

$\dot{M}O_2$ was not recorded during the swimming trails, due to the low biomass per water volume combined with already low $\dot{M}O_2$ rates at low water temperatures. Therefore, the swim tunnel was used in open mode with an aquarium pump (550 L h^{-1}) constantly replenishing the water in the swim tunnel with oxygen saturated water from the surrounding reservoir tank, that was aerated with compressed air. The water temperature of the surrounding tank was constantly monitored with a WTW LF 97 multimeter.

Maximum and standard metabolic rate

The setup for respiration measurements (MMR_{ex}, SMR, MMR_{ex+dig}) subsequent to the exhaustion protocol was identical to the one described above for the recording of the full SDA response and period. For all treatments, $\dot{M}O_2$ measurement periods of 60 min altered with flush periods of 30 min with an exception for the treatment 6°C/fed (45 min measurement: 15 min flush). Background respiration was recorded subsequent to each measurement round in one representative respiration chamber for several hours.

Immediately after reaching their critical swimming speed, the fish were weighed and transferred from the swim tunnel to respiration chambers in order to determine their MMR_{ex} (unfed fish), as well as the MMR_{ex+dig} (fed fish) defined as the first recorded $\dot{M}O_2$ value. For recovery, the fish remained in the respiration chambers for approx. 48 h. The 20% quantile of $\dot{M}O_2$ data starting 24h after placement in the respiration chamber was considered as the individual standard metabolic rate (SMR), a method suggested by Chabot et al. (2016b). As the same individuals were used consecutively as unfed and fed fish in an identical experimental setup, SMR was only determined in the unfed fish.

Statistical analysis

All $\dot{M}O_2$ data were normalized for average fish weight (14.0 g) as suggested by Steffensen et al. (1994). The energetic efficiency of maximum swimming performance (E_{max}) was calculated as the ratio U_{crit} MMR^{-1}.

The individual SDA signals were analyzed by aid of a specific script for SDA calculation (Chabot et al. 2016a) in the program R version 3.0.2 (R Core Team 2013) using the packages *quantreg* (Koenker 2015) and *Hmisc* (Harrell 2015). With the aid of this script a curve describing the SDA response over time was generated enabling the quantification of the following SDA variables: (1) amplitude of peak $\dot{M}O_2$ (MMR_{dig}) and (2) aerobic scope of digestion (AS_{dig}) calculated as the difference between MMR_{dig} and SMR. The (3) duration of the SDA response is characterized by the intercept of the fitted curve with the SMR baseline. The integral below the curve describes the total post-prandial increase in $\dot{M}O_2$ and, thereby, characterizes the (4) magnitude of the individual SDA response (Chabot et al. 2016a).

SMR was classified as the 20% quantile among $\dot{M}O_2$ values obtained on the 18th day (0°C) or the 9th day (6°C) subsequent to the termination of the SDA response. By then, $\dot{M}O_2$

values had clearly reached a baseline level and SMR records were comparable to those recorded for post-prandial individuals after the exercise test.

Previous tests had shown that elevated $\dot{M}O_2$ rates due to handling stress decreased to SMR within the first hour after transfer into the respiration chamber. To prevent an impact of handling stress on the calculation of SDA variables, we excluded the initial data point (1 h).

According to suggestions of Chabot et al. (2016a), tau (τ) was set to 0.2 to allow 20% of the $\dot{M}O_2$ observations to fall below the curve describing the SDA response over time. For the flexibility parameter lambda (λ) of the curve, default settings were chosen (Chabot et al. 2016a). A tolerance limit of 0.05% of SMR was added to the calculated value of SMR to determine the SDA duration (Chabot et al. 2016a).

Statistical analysis in the present study served to compare effects of acclimation temperature and feeding status on respiratory variables and swimming performance. Further, the same variables were compared after acute exposure to an intermediate temperature.

All tests were performed using the program SigmaPlot 13 (Systat Software Inc, San Jose, California, USA). Prior to statistical analysis, individuals that clearly refused to feed were excluded from the SDA data set (0°C: n = 1; 6°C: n = 2). Further, data obtained from individuals which refused to swim (0°C/fed: n = 1; 0°C/unfed: n = 3) were removed from the variables U_{gait}, U_{crit}, MMR and E_{max}. One additional individual (6°C/fed) clearly remained in the corner of reduced current during the swimming trial and therefore showed distinctly higher values for U_{gait} and U_{crit}. These unrepresentative U_{gait} and U_{crit} values were removed from the respective data sets. One further U_{crit} value was removed from statistical analysis (0°C/unfed) for better comparison with the variable U_{gait} due to lacking burst activity of the respective individual.

For the comparison of SDA responses between temperatures, as well as for the detection of effects of acclimation temperature and feeding status on respiratory (SMR, MMR, AS) and swimming performances (U_{gait}, U_{crit}, E_{max}, maximum BC (max. BC)) of *B. saida*, a set of t-tests was performed. T-tests required normal distribution, which was confirmed with a Shapiro-Wilk test. Homoscedasticity was determined with Levene tests. In case of

heteroscedasticity (compare Table 2 - 4), Mann-Whitney Rank Sum tests were performed. A significance level of $p < 0.05$ was accepted.

All graphs were created in the program R version 3.0.2 (R Core Team 2013).

Results

The datasets generated and analysed during the current study are available in Kunz et al. (2018a) (https://doi.pangaea.de/10.1594/PANGAEA.889161).

A summary of mean values for each parameter and treatment is presented in Table 1. The results of the t-tests are listed in Table 2 (SDA parameter), Table 3 (respiration and swimming performance) and Table 4 (metabolic performance evoked by digestion and exercise).

At the same temperatures, all fish consumed similar daily rations (Table 1). However, the amount of voluntary food intake was higher at 6°C than at 0°C.

SDA

The mean values of both SMR and MMR_{dig} were elevated at 6°C compared to the control treatment at 0°C (SMR: t = -1.520, p = 0.167, MMR_{dig}: t = -3.786, p = 0.013). However, the increase in SMR with acclimation temperature was less pronounced compared to the increase in MMR_{dig} (Fig. 1), resulting in a non-significantly enhanced aerobic metabolic scope of digestion (AS_{dig}) (t = 1.699, p = 0.150) at 6°C. SDA duration was not affected by temperature (t = 0.610, p = 0.568). The total SDA magnitude (amount of excess oxygen consumed), however, was non-significantly elevated in the warmth (t = -0.302, p = 0.775) (Fig. 1).

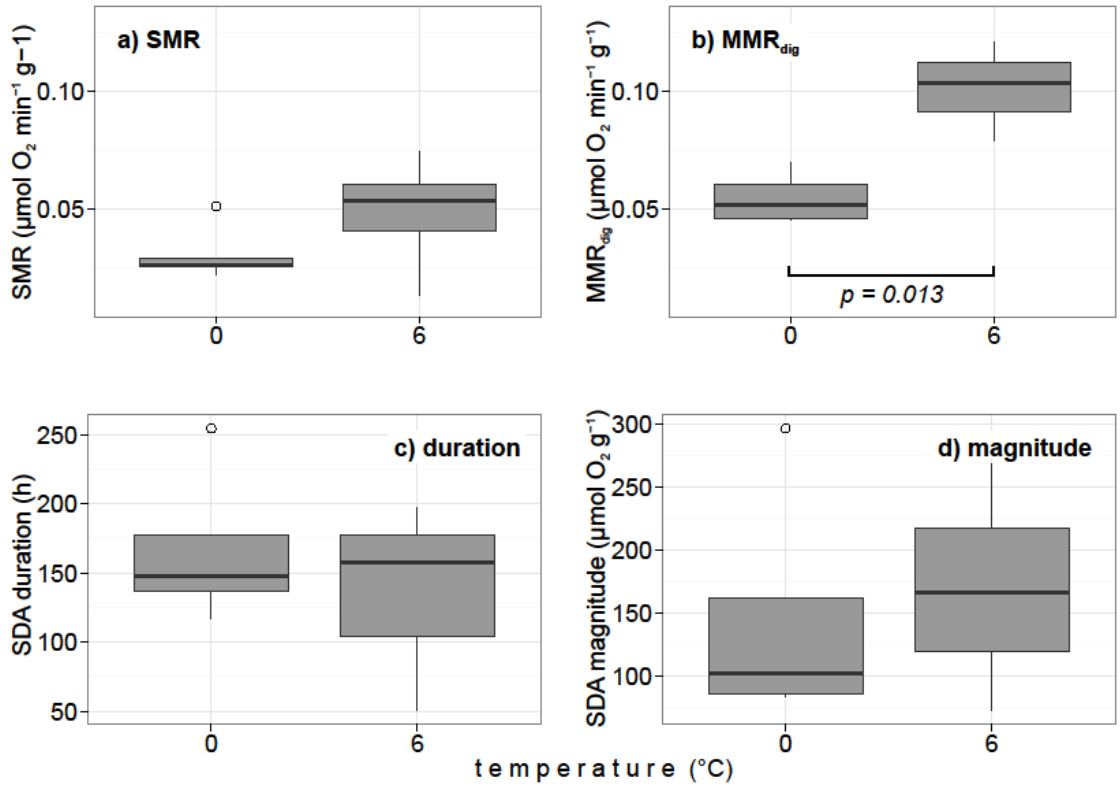

Fig. 1 Specific dynamic action (SDA) response. a) standard metabolic rate (SMR) (µmol O_2 min^{-1} g^{-1}) (n = 5 at 0 and 6°C, respectively), b) maximum metabolic rate evoked by digestion (MMR_{dig}) (µmol O_2 min^{-1} g^{-1}) (n = 4 at 0°C, n = 3 at 6°C), c) SDA duration (h) (n = 4 at 0°C, n = 3 at 6°C), d) SDA magnitude (µmol O_2 g^{-1}) (n = 4 at 0°C, n = 3 at 6°C). The p-value represents statistically significant differences between temperature-treatments (compare Table 1)

Maximum and standard metabolic rate

The SMR of long-term acclimated *Boreogadus saida* was not affected by temperature (t = -1.166, p = 0.265). In contrast, the MMR_{ex} of food-deprived fish rose with temperature (t = -2.241, p = 0.049) (Fig. 2). Accordingly, a (non-significantly) elevated aerobic scope for exercise (AS_{ex}) (t = -1.887, p = 0.088) was recorded at 6°C (Fig. 3, black bars). It should be noted, that the MMR_{ex} of several unfed individuals (n = 3 out of total n = 7) could not be determined at 0°C as these specimens refused to swim.

In fed individuals, the increase in maximum metabolic rate (MMR_{ex+dig}) with temperature was more pronounced (t = -9.218, p < 0.001) than in unfed *B. saida*. Consequently,

MMR_{ex+dig} was higher compared to MMR_{ex} (t = 2.509, p = 0.025) in the warmth. No such trend was visible between fed and food-deprived fish at 0°C (t = -0.695, p = 0.507) (Fig. 2). During acute exposure to 3°C (further abbreviated as 3°C/0°C and 3°C/6°C for the cold- and warm-acclimated group, respectively), MMR_{ex+dig} revealed intermediate values (0°C versus 3°C/0°C, t = -3.804, p = 0.003; 6°C versus 3°C/6°C, t = 5.191, p < 0.001) with a higher MMR_{ex+dig} in the cold-acclimated group (3°C/0°C versus 3°C/6°C, t = 1.732, p = 0.109) (Fig. 2).

The MMR_{dig} caused by digestion alone was lower than the MMR_{ex} attained during exercise (t = -4.598, p = 0.004) as well as the MMR_{ex+dig} attained during combined exercise and digestion (t = -5.031, p = 0.001) at 0°C. At 6°C, this difference was only apparent between MMR_{dig} and MMR_{ex+dig} (U = 1.000, p = 0.024).

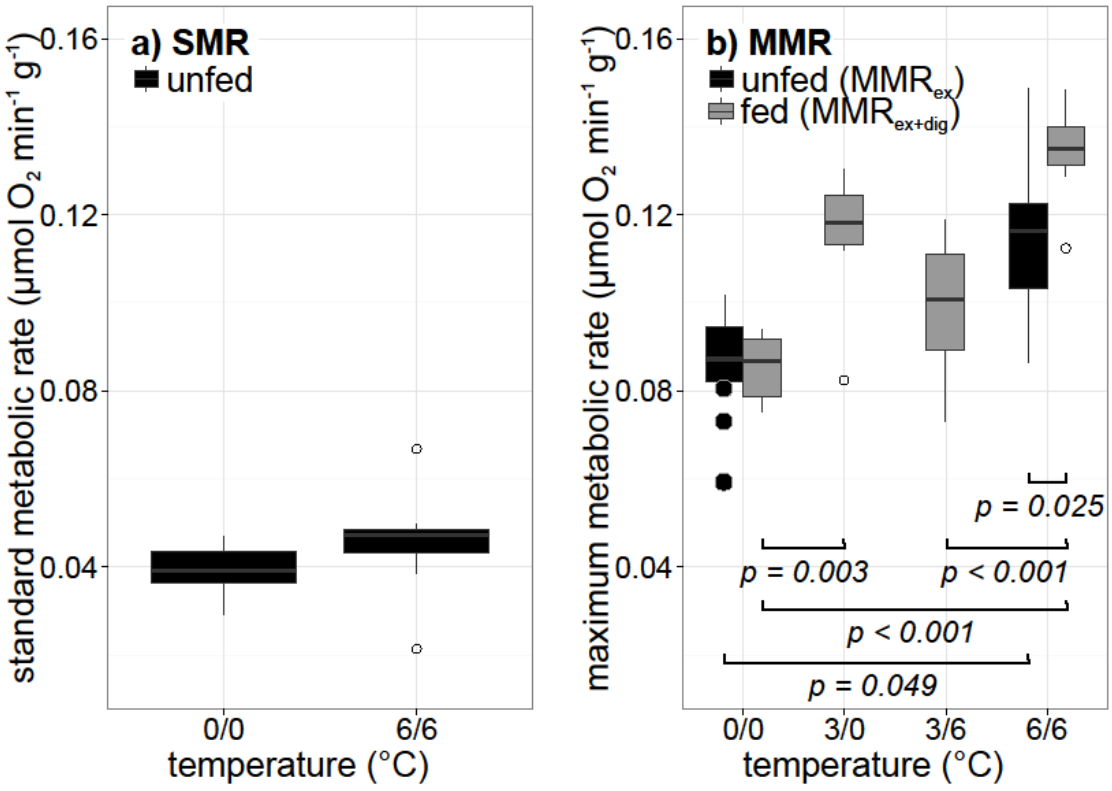

Fig. 2 a) SMR (n = 7 at 0°C, n = 8 at 6°C) and b) maximum metabolic rate obtained subsequent to exercise (MMR_{ex}) (n = 4 at 0°C, n = 8 at 6°C) and subsequent to combined exercise and digestion (MMR_{ex+dig}) (µmol O_2 min^{-1} g^{-1}) (n = 6 at 0°C, n = 6 at 3/0°C, n = 8 at 3/6°C, n = 8 at 6°C). X-axis shows experimental temperature (ET) and acclimation temperature (AT) (ET/AT). Solid points represent MMR-values obtained from individuals that refused swimming during the exercise protocol (excluded from statistical analysis). *P*-

values represent statistically significant differences between temperature-treatments and between feeding-treatments, respectively (compare Table 2)

Aerobic scope

A significant temperature-effect on aerobic scope was solely detected in fed exercised *B. saida* (t = -5.540, p < 0.001) (Fig. 3).

The aerobic scope recorded in fed individuals during exercise (AS_{ex+dig}) at 0°C did not reveal significant differences from separate determinations of aerobic scope of digestion (AS_{dig}) (t = -2.212, p = 0.058) and of exercise (AS_{ex}) (t = -0.472, p = 0.650). Neither AS_{dig} and AS_{ex} (t = -2.286, p = 0.062) showed significant differences from each other in the cold (Fig. 3).

At 6°C, however, the simultaneous metabolic needs of digestion and exercise caused a higher aerobic scope compared to AS_{dig} (t = -4.675, p = 0.001) and AS_{ex} (t = 2.361, p = 0.033), respectively. Furthermore, AS_{dig} was lower than AS_{ex} (t = -2.360, p = 0.043) at the warm acclimation temperature (Fig. 3).

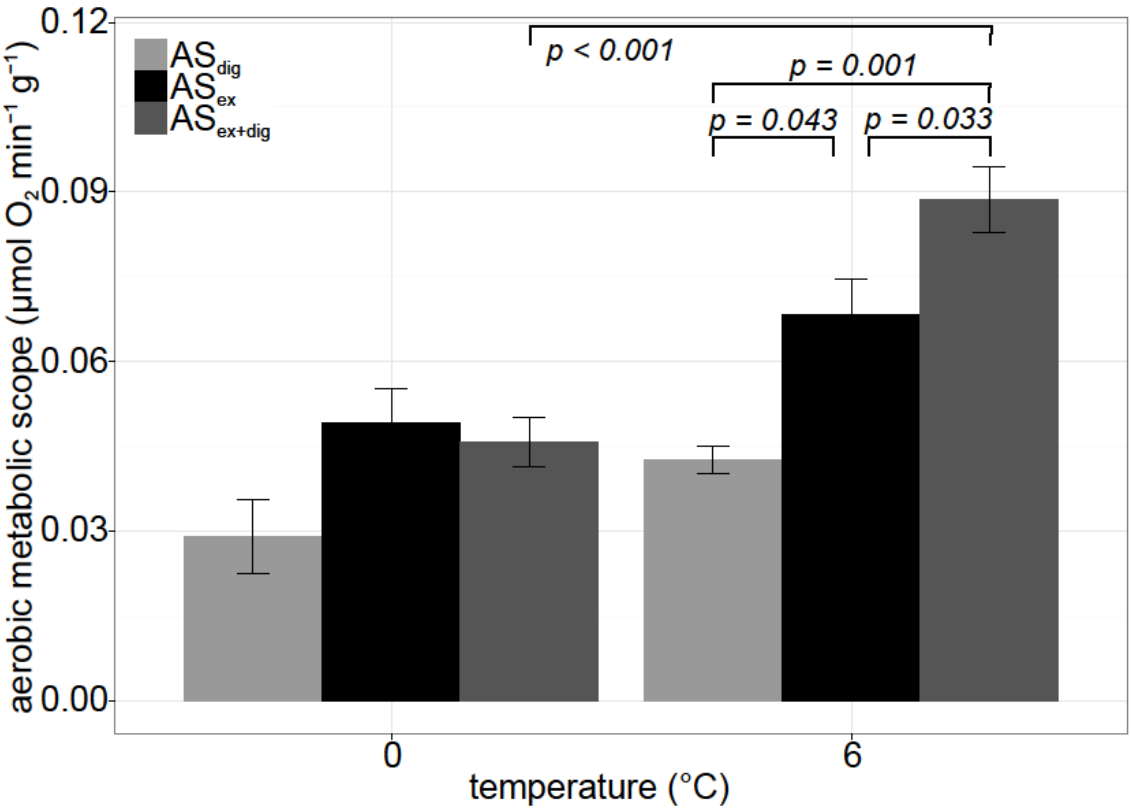

Fig. 3 Comparison of aerobic metabolic scope obtained by digestion (AS_{dig}) (n = 4 at 0°C, n = 3 at 6°C), by exercise of unfed individuals (AS_{ex}) (n = 4 at 0°C, n = 8 at 6°C) and by exercise of fed individuals (AS_{ex+dig}) (µmol O_2 min^{-1} g^{-1}) (n = 6 at 0°C, n = 8 at 6°C). P-values indicate statistically significant differences between AS_{dig} and AS_{ex+dig}, and AS_{ex} and AS_{ex+dig}, respectively, at each temperature

Swimming performance

Solely among fed individuals, the swimming performance was positively correlated with temperature (fed: 0°C versus 6°C, t = -7.447, p < 0.001 (U_{gait}), 0°C versus 6°C, t = -6.286, p < 0.001 (U_{crit}); unfed: 0°C versus 6°C, t = -1.394, p = 0.197 (U_{gait}), 0°C versus 6°C, t = -0.673, p = 0.518 (U_{crit})) (Fig. 4). This resulted in significantly higher values of U_{gait} (t = 5.076, p < 0.001) and U_{crit} (t = 3.781, p = 0.002) in fed fish than in unfed fish at the warm acclimation temperature. However, the energetic efficiency of swimming performance (E_{max}, metabolic demand at U_{crit}) was lower in fed fish at 6°C than at 0°C (t = 3.016, p = 0.011).

At 0°C, U_{gait} and U_{crit} were (non-significantly) reduced in fed fish compared to unfed fish at 0°C (t = -1.074, p = 0.318 and U = 3.000, p = 0.167, respectively). However, three out of seven individuals refused to swim unfed in the cold. No such behavior appeared at 6°C under food deprivation. After consecutive short-term exposure to 3°C, fed *B. saida* showed an intermediate U_{gait} (0°C versus 3°C/0°C, t = -2.820, p = 0.018; 6°C versus 3°C/6°C, U = 8.000, p = 0.021) and U_{crit} (0°C versus 3°C/0°C, t = -2.432, p = 0.035; 6°C versus 3°C/6°C, t = 3.493, p = 0.004) with both parameters being unaffected by the initial acclimation temperature (3°C/0°C versus 3°C/6°C, t = 0.497, p = 0.628 (U_{gait}); t = 0.350, p = 0.733 (U_{crit})) (Fig. 4). However, E_{max} was reduced in cold-acclimated *B. saida* swimming acutely at a warmer temperature (t = 1.994, p = 0.074), while short-term cooling caused an increase in E_{max} after acclimation to 6°C (U = 14.000, p = 0.065).

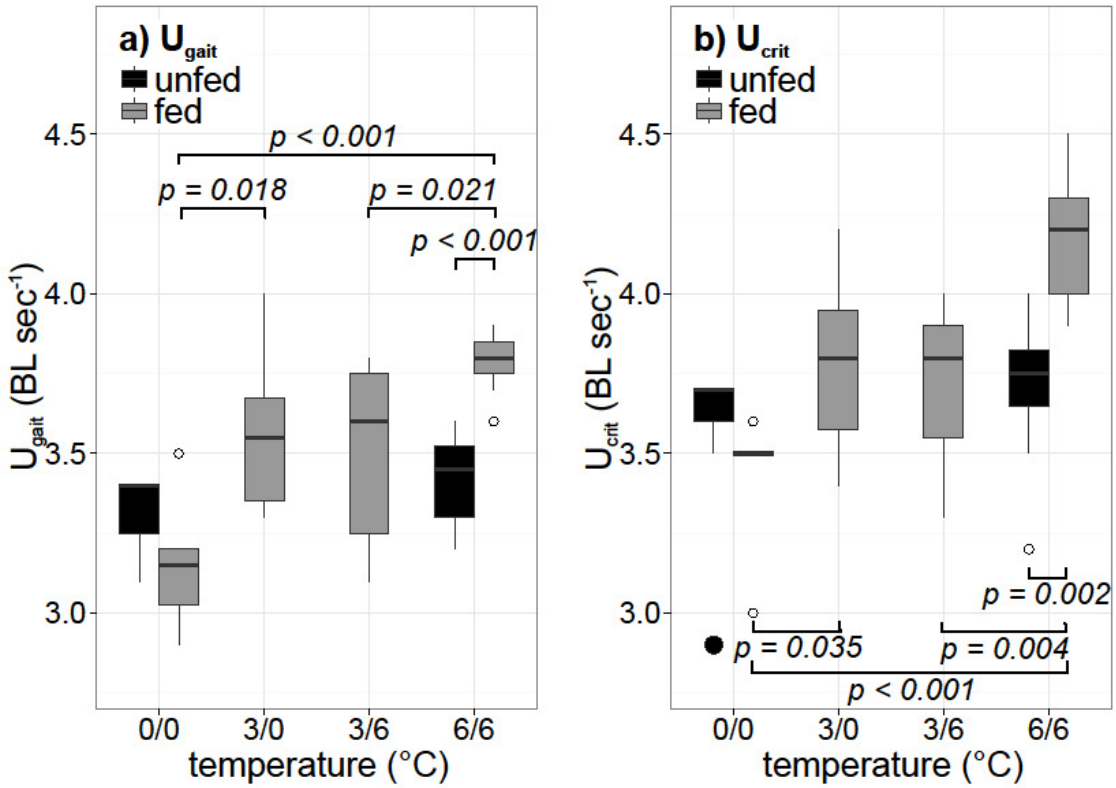

Fig. 4 a) Gait transition (U_{gait}) (unfed: n = 3 at 0°C, n = 8 at 6°C; fed: n = 6 at 0°C, n = 6 at 3/0°C, n = 8 at 3/6°C, n = 7 at 6°C) and b) critical swimming speed (U_{crit}) (BL sec^{-1}) (unfed: n = 3 at 0°C, n = 8 at 6°C; fed: n = 6 at 0°C, n = 6 at 3/0°C, n = 8 at 3/6°C, n = 7 at 6°C). X-axis shows experimental temperature (ET) and acclimation temperature (AT) (ET/AT). Solid points represent specimens without burst capacity (excluded from statistical analysis). *P*-values represent statistically significant differences between temperature-treatments and between feeding-treatments, respectively (compare Table 2)

The maximum number of burst swimming events of thermally acclimated fish did not show any temperature trend. Food deprivation, however, caused a non-significantly reduction in the maximum number of bursts when compared to the burst performance of fed fish (0°C, t = 0.939, p = 0.375; 6°C, U = 12.500, p = 0.072). Acute exposure of fed fish to an intermediate temperature of 3°C revealed a slightly elevated maximum number of bursts in the cold-acclimated group (t = -1.282, p = 0.229) (Fig. 5).

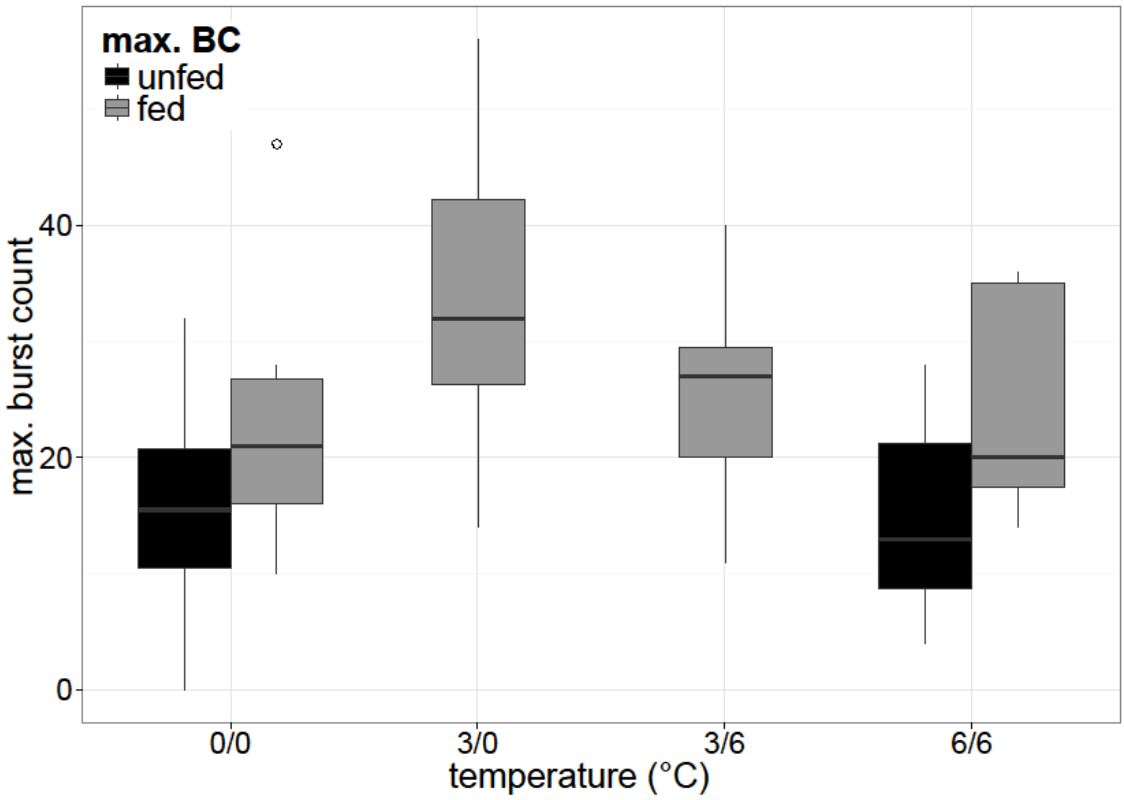

Fig. 5 Maximum burst count (max. BC) (unfed: n = 4 at 0°C, n = 8 at 6°C; fed: n = 6 at 0°C, n = 6 at 3/0°C, n = 8 at 3/6°C, n = 7 at 6°C). X-axis shows experimental temperature (ET) and acclimation temperature (AT) (ET/AT). No statistically significant differences were found between temperature-treatments and between feeding-treatments, respectively (compare Table 2)

Discussion

In the present study, we investigated the energy allocation to exercise and digestion at ambient (0°C) and projected elevated (6°C) water temperatures in the Arctic key species *Boreogadus saida*. We further examined potential impacts of thermal acclimation history on the maximum performance capacity associated with simultaneous exercise and digestion at an intermediate temperature (3°C).

The SMR in the present study is comparable to published $\dot{M}O_2$ values for *B. saida* from the Canadian Arctic (Hop and Graham 1995; Drost et al. 2016). In line with our findings from the SDA experiment, Drost et al. (2016) found a higher SMR at 6°C than at 0°C. Low metabolic rates at cold habitat temperatures are considered essential for efficient growth during periods of prey availability and for restricted weight loss during food shortage (Hop and Graham 1995). However, a positive correlation between SMR and temperature was not found in *B. saida* used for swimming experiments, indicating full metabolic compensation at 6°C (type II compensation, Precht 1958). This discrepancy between our experimental findings is possibly due to a shorter acclimation time of the "SDA group" (13 days) compared to the "exercise group" (45 days) at 6°C. Kunz et al. (2018b) also detected full metabolic compensation in *B. saida* after an acclimation period of four months to 6°C. Thus, *B. saida* may have the capacity to balance the baseline costs for vital functions after long-term acclimation to 6°C.

The level of MMR_{dig} was approximately two-fold SMR at both 0°C and 6°C. The only previous study investigating the SDA response of *B. saida* of similar size (14.4 g) found a peak $\dot{M}O_2$ of only 1.3 times the value of SMR and thereby a distinctly lower (15.8%) MMR_{dig} at 0.4°C (Hop and Graham 1995). Although MMR_{dig} is generally known to increase with meal size (Jobling 1981), *B. saida* in the study of Hop and Graham (1995) consumed a 4.4-fold higher quantity of food (15% BW) (frozen krill) with a composition similar to the diet in the present study (3.4% BW). The feeding protocol in both studies was fairly similar with individuals being allowed to feed voluntarily on two (Hop and Graham 1995) and four (present study) consecutive days prior to the $\dot{M}O_2$ measurement. This method ensured satiation and, thereby, measurement of the maximum possible $\dot{M}O_2$ after digestion (Soofiani and Hawkins 1982), while preventing overestimations in metabolic rate due to handling stress as evoked by force-feeding. Therefore, the distinct difference in meal size between both studies may potentially be caused by divergent procedures for the

determination of ration size (information not provided in Hop and Graham (1995)) or different feeding histories. A comparison of meal sizes hence cannot explain the difference in MMR_{dig} in this specific case. Yet, a two-fold increase in metabolic rate above SMR due to digestion as recorded in the present experiment is well within the upper range of MMR_{dig} of various other species (1.6 – 2.0 times SMR) (Jobling 1981). Furthermore, a high MMR_{dig} can be expected for juvenile specimens that possess high growth potential, as the SDA response is considered to reflect metabolic investment in protein synthesis and deposition and, thereby, the metabolic cost of growth (Brown and Cameron 1991b).

MMR_{dig} of *B. saida* increased with temperature more than SMR resulting in an elevated (non-significantly) aerobic scope of digestion (AS_{dig}) at 6°C. A higher AS_{dig} at 6°C indicates a larger investment of *B. saida* into growth and thus represents a precondition for improved growth performance at 6°C, in line with the results of a growth study of *B. saida* from the same area (Kunz et al. 2016). A temperature-effect on MMR_{dig} also involved a larger meal size consumed at 6°C, which may – at least partly – have triggered the observed increase in metabolic rate (Muir and Niimi 1972; Beamish 1974).

SDA duration of *B. saida* did not reveal a significant correlation with temperature. In general, however, the duration of the post-prandial $\dot{M}O_2$ pattern has been shown to decrease with rising temperature (e.g. Saunders 1963; Jobling and Spencer Davies 1980) due to accelerated biochemical processes and increased intestinal peristalsis that result in faster gastric evacuation (Jobling and Spencer Davies 1979) and gut passage rates (Beamish 1974). Shorter gastric retention times in the warmth allow a higher food uptake as observed in the present study. In fact, the larger amount of food - also reflected in the (non-significantly) elevated SDA magnitude at 6°C - likely counteracted the theoretical reduction in SDA duration in the warmth (Jobling and Spencer Davies 1980).

The MMR_{ex} of unfed fish was lower than published values for *B. saida* originating from the same population from Svalbard (0.1094 µmol O_2 min^{-1} g^{-1}, 0°C; 0.1409 µmol O_2 min^{-1} g^{-1}, 6°C) (Kunz et al. 2018b) and from the Canadian Arctic (~ 0.1517 µmol O_2 min^{-1} g^{-1}, 1°C; ~ 0.2150 µmol O_2 min^{-1} g^{-1}, 6.5°C; Drost et al. 2016), with $\dot{M}O_2$ values adjusted to the average fish weight of the present study. The deviant values for MMR_{ex} might be attributed to a longer period of food deprivation (0°C: 18 days, 6°C: 9 days) and a higher degree of gastric evacuation in the present study compared to Drost et al. (2016) (2 days)

and Kunz et al. (2018b) (4 days). Thus, the SDA signal of fish in the two latter studies may not have been completely finished by the time of MMR_{ex} measurements.

In line with results found for various fish species (e.g. Atlantic cod *Gadus morhua* (Claireaux et al. 2000) and European sea bass *Dicentrarchus labrax* (Claireaux and Lagardère 1999)), the MMR_{ex} of unfed *B. saida* increased significantly with acclimation temperature. MMR_{ex} rising with temperature are likely evoked by the thermally induced stimulation of biochemical processes (e.g. Taylor et al. 1997), also causing higher maximum heart rates during exercise after warm-acclimation as found for *B. saida* acclimated to 0.5 – 6.5°C (Drost et al. 2016). As a result of compensated SMR and elevated MMR_{ex} we found the metabolic aerobic scope for exercise (AS_{ex}) of acclimated *B. saida* to increase 1.4-fold between 0 and 6°C. However, despite the higher AS_{ex} in the warmth, swimming performance (U_{gait} and U_{crit}) of unfed fish did not increase at 6°C in line with previous findings for *B. saida* from Svalbard (Kunz et al. 2018b). This indicates that the energy utilization efficiency of *B. saida* decreases after warm-acclimation when operating at maximum metabolic capacity.

At 6°C, fed *B. saida* showed an elevated MMR_{ex+dig} compared to MMR_{ex}. The capacity to elevate the MMR_{ex+dig} above the MMR_{ex} has been interpreted as evidence for simultaneous aerobic processes of locomotion and digestion in some species and therefore as a precondition for maintaining the maximum swimming speed even during parallel energetic demands from digestion (*D. labrax* at 20°C, Jourdan-Pineau et al. 2009; darkbarbel catfish *Peltebagrus vachelli* at 25°C, Li et al. 2010; goldfish *Carassius auratus*, common carp *Cyprinus carpio* and qingbo *Spinibarbus sinensis*, all at 15°C, Pang et al. 2011). However, we found both U_{gait} and U_{crit} to be significantly higher in fed than in unfed fish at 6°C, indicating that food deprivation caused a shortage in energy-supply resulting in muscular capacities not being fully exploited in unfed individuals at this temperature. In contrast, the higher swimming performance in fed *B. saida* might be evoked by dietary amino acids, available in excess to those used for protein biosynthesis, and their utilization for immediate energy production (Alsop and Wood 1997).

Unlike the situation at warm acclimation temperatures, we found MMR at U_{crit} to not differ between feeding treatments at 0°C, suggesting a limit of cardio-respiratory capacities to constrain food-induced metabolic stimulation in the cold (Alsop and Wood 1997). Accordingly, AS_{ex} and AS_{ex+dig} were almost equal at 0°C and aerobic swimming capacity

in fed *B. saida* (non-significantly) reduced compared to unfed fish. The elevated O_2 demand in the gastrointestinal tissue may constrain energy expenditure for exercise at 0°C. This trade-off has also been observed in *Oncorhynchus mykiss* (Alsop and Wood 1997), *Oncorhynchus tshawytscha* (Thorarensen and Farrell 2006) and *C. auratus* (25°C) (Pang et al. 2011; Zhang et al. 2012). Hence, the elevated visceral blood flow owing to digestive processes cannot arbitrarily be redistributed to red muscle during exercise at 0°C, likely resulting in O_2–supply limitations in red muscle tissue that impair swimming capacity (Thorarensen and Farrell 2006).

The anaerobic swimming capacity of *B. saida* – measured as burst events – was not influenced by acclimation temperature in both food-deprived and fed fish, in line with the results for burst performance of the same species evoked in a U_{crit} protocol with acclimation temperatures between 0 and 8°C (Kunz et al. 2018b). This may indicate a low thermal sensitivity in the glycolytic ATP production (Sidell and Moerland 1989) and in the use of white muscle glycogen stores (compare review by Kieffer 2000). Further, the absence of a temperature-effect on anaerobic swimming might be attributed to an overall low capacity of anaerobic metabolism in *B. saida* compared to temperate species characterized by a more active lifestyle (e.g. *D. labrax*, Marras et al. 2010). Likewise, constraints on burst performance are also discussed for cold-adapted Antarctic notothenoids that revealed low glycolytic capacity in response to exhaustive exercise (Dunn and Johnston 1986; Davison et al. 1988).

However, maximum burst performance was non-significantly elevated in fed compared to unfed individuals at both temperatures. During anaerobic exercise, white muscle glycogen stores are known to be exploited (e.g. Wood 1991). The limited burst activity in unfed fish in our study may therefore be explained by reduced glycogen stores, similar to findings for food-deprived *O. mykiss* acclimated to 15°C (Scarabello et al. 1991; Kieffer and Tufts 1998). Further, glycogen stores have been shown to be more sensitive to food deprivation in juvenile fish, as considered in our study, than in adults (Kieffer and Tufts 1998).

Three individuals (out of n = 7) refused to swim when unfed at 0°C, while the same individuals showed normal swimming behavior after feeding. One further specimen did not display any anaerobic swimming capacity in the unfed state at 0°C. The absence of (anaerobic) swimming capacity in these particular individuals following food deprivation indicates limited use or availability of energy stores in muscle tissue in the cold.

Burst-type exercise is generally accepted as a swimming mode essential for escape reactions during predator-prey encounters as well as during foraging (Beamish 1978). In *B. saida*, anaerobic metabolism contributes little to the overall swimming capacity (Kunz et al. 2018b), indicating a low ecological relevance of burst performance in this species as discussed for *Notothenia neglecta* (Dunn and Johnston 1986). While camouflage has been proposed as a predator-avoidance strategy in *N. neglecta* (Dunn and Johnston 1986), *B. saida* is reported to outlast predator encounters in ice crevices (Lønne and Gulliksen 1989). Further, *B. saida* prey upon rather slow organisms such as amphipods and copepods (Lønne and Gulliksen 1989) in upper water layers that are associated with high prey density (David et al. 2015), thereby likely relying mainly on aerobic swimming performance.

The energetic requirements for digestion were found to be lower than for exercise in *B. saida* at both 0 and 6°C. As stated above, costs for SDA are accepted to represent the energetic investment for growth (Brown and Cameron 1991b) and, therefore, are more pronounced in fast growing species with a large body length (e.g. *G. morhua*), in order to minimize the time span of vulnerable body size (Hunt von Herbing and White 2002). *Boreogadus saida*, however, is known to have a rather low growth potential (Laurel et al. 2015; Kunz et al. 2016) with a maximum body size of only ~ 30 cm (Falk-Petersen et al. 1986).

Furthermore, *B. saida* has been reported to lead a moderately active or even sluggish lifestyle (Gradinger and Bluhm 2004). In this context, it is surprising, that aerobic metabolic scope for sustainable locomotion is relatively high over a wide range of habitat temperatures in *B. saida*. To our knowledge, the ecological need of *B. saida* for sustainable swimming capacity has not yet been described. A recent study, however, raised the hypothesis of larval *B. saida* drifting passively with sea-ice from coastal hatching areas to the central Arctic (David et al. 2015), implying the need for migrating back to spawning areas. Assuming *B. saida* to perform spawning-migrations might be an explanation for the metabolic prioritization of aerobic swimming performance.

We further investigated the aerobic performance of cold- and warm-acclimated *B. saida* during acute exposure to 3°C in order to identify potential differences between groups at 0 and 6°C due to thermal acclimation. Short-term exposure to an intermediate temperature (3°C) evoked a higher MMR_{ex+dig} in cold-acclimated *B. saida* fed to satiation than in warm-acclimated specimens despite lower food intake in the former group. Accordingly, the

impact of digestion fails to explain the difference in MMR_{ex+dig} in this particular case. $\dot{M}O_2$, however, is highly dependent on cardiac performance (Priede and Tytler 1977). In fact, *B. saida* acclimated for six months to 0.5°C showed higher maximum heart rates compared to warm-acclimated *B. saida* (6.5°C), when acutely exposed to 3°C (~ 32 bpm and ~ 27 bpm, respectively) (Drost et al. 2016). This may explain the elevated MMR_{ex+dig} in the cold acclimated group at 3°C seen in the present study.

Despite (non-significantly) higher MMR_{ex+dig} in cold-acclimated individuals, no difference was detected in the swimming performance (U_{gait} and U_{crit}) between cold- and warm-acclimated *B. saida* at 3°C indicating reduced swimming efficiency in the acutely warmed cold-acclimated group. The difference in MMR_{ex+dig} between acclimation groups suggests that maximum aerobic capacities of *B. saida* were partly compensated after acclimation to 6°C. In contrast, the comparable swimming performance of cold and warm-acclimated *B. saida* under short-term exposure to 3°C provides evidence that thermal acclimation of muscular capacity did not occur.

In conclusion, the present study revealed that aerobic and anaerobic capacities of *Boreogadus saida* strongly depend on its feeding status, especially at the projected elevated acclimation temperature. Food availability in the Arctic, however, is expected to decrease for this species during the ongoing rise in seawater temperature and the associated decline in sea-ice habitat which represents a rich feeding ground for juvenile *B. saida* (David et al. 2015). Reduced prey abundance and increasing metabolic demands in warmer waters will likely force *B. saida* to put more effort into foraging. Therefore, the inability to exploit the full potential of aerobic swimming muscle capacities under food deprivation at 6°C concomitant with a reduced energy utilization efficiency may indicate a decrease in competitive strength of *B. saida* in the warmth. Especially during more frequent encounters with eurythermal temperate species like *Gadus morhua*, food-restricted *B. saida* can be expected to suffer from enhanced predation (Renaud et al. 2012). Likewise, further invading temperate species of similar ecotype as *B. saida* such as Atlantic herring *Clupea harengus* and capelin *Mallotus villosus*, can be expected to be increasingly successful when competing with *B. saida* for both food and space in a warming Arctic (Hop and Gjøsæter 2013).

Acknowledgements

This study was part of the research program BIOACID (Biological Impacts of Ocean Acidification, phase II), funded by the German Federal Ministry of Education and Research (BMBF, WP 4.1 and 4.2, FKZ 03F0655B). KLK, HOP, RK and FCM acknowledge funding through the PACES (Polar Regions and Coasts in a Changing Earth System) program of the Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI). We gratefully acknowledge the crew of RV *Heincke* (AWI) (HE 451.1) for animal collection. Further, we would like to thank Fredy Véliz Moraleda, Pia Graulich and Hanna Scheuffele for their support during the experimental setup and during animal maintenance.

Compliance with Ethical Standards

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed and approved under reference number 522-27-11/02-00 (113).

This article does not contain any studies with human participants performed by any of the authors.

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Tables

Table 1 Summary of results (means ± SEM). SMR = standard metabolic rate, MMR_{dig} = maximum $\dot{M}O_2$ obtained during digestion, AS_{dig} = aerobic scope of digestion, duration = time span of SDA response, magnitude = area underneath the curve that is described by the post-prandial increase in $\dot{M}O_2$ values, U_{gait} = gait transition speed, U_{crit} = critical swimming speed, max. BC = maximal burst count, MMR_{ex} = maximum $\dot{M}O_2$ obtained subsequent to U_{crit}, AS_{ex} = absolute aerobic scope, E_{max} = energetic efficiency of maximum swimming performance, MMR_{ex+dig} = maximum $\dot{M}O_2$ obtained during simultaneous exercise and digestion. Number of individuals per variable is shown in brackets in case it differs from the whole treatment individual number

Digestion	*Temperature (°C)*	
	0 (n = 5)	6 (n = 5)
wet weight (g)	11.7 ± 0.9	8.5 ± 1.1
total length (cm)	11.8 ± 0.3	10.9 ± 0.4
daily ration (% BW)	3.4	4.4
SMR (µmol O_2 min^{-1} g^{-1})	0.0306 ± 0.0052	0.0484 ± 0.0104
MMR_{dig} (µmol O_2 min^{-1} g^{-1})	0.0545 ± 0.0058 (n = 4)	0.1012 ± 0.0123 (n = 3)
AS_{dig} (µmol O_2 min^{-1} g^{-1})	0.0290 ± 0.0065 (n = 4)	0.0426 ± 0.0024 (n = 3)
duration (h)	166.5 ± 30.2 (n = 4)	135.3 ± 43.9 (n = 3)
magnitude (µmol O_2 g^{-1})	146.16 ± 50.59 (n = 4)	169.14 ± 56.27 (n = 3)
Exercise	*Temperature (°C)*	
	0 (n = 7)	6 (n = 8)
wet weight (g)	13.8 ± 1.5	13.1 ± 0.7
total length (cm)	12.9 ± 0.4	13.0 ± 0.2
U_{gait} (BL sec^{-1})	3.3 ± 0.1 (n = 3)	3.4 ± 0.1
U_{crit} (BL sec^{-1})	3.6 ± 0.1 (n = 3)[a]	3.7 ± 0.1
max. BC	15.8 ± 6.6 (n = 4)	15.0 ± 3.0
SMR (µmol O_2 min^{-1} g^{-1})	0.0393 ± 0.0024	0.0455 ± 0.0045
MMR_{ex} (µmol O_2 min^{-1} g^{-1})	0.0892 ± 0.0049 (n = 4)	0.1137 ± 0.0072
AS_{ex} (µmol O_2 min^{-1} g^{-1})	0.0492 ± 0.0060 (n = 4)	0.0683 ± 0.0064
E_{max} (BL g $µmol^{-1}$)	2334.8 ± 214.3 (n = 4)	2008.0 ± 134.3

Exercise & digestion	*Temperature (°C)*			
	0 (n = 7)	3/0 (n = 6)	3/6 (n = 8)	6 (n = 8)
wet weight (g)	16.4 ± 1.4	16.6 ± 1.4	16.6 ± 1.2	13.4 ± 0.8
total length (cm)	13.2 ± 0.3	13.3 ± 0.4	13.1 ± 0.2	12.5 ± 0.2
daily ration (% BW)	2.6	2.6	4.3	4.2
U_{gait} (BL sec^{-1})	3.2 ± 0.1 (n = 6)	3.6 ± 0.1	3.5 ± 0.1	3.8 ± 0.0 (n = 7)
U_{crit} (BL sec^{-1})	3.4 ± 0.1 (n = 6)	3.8 ± 0.1	3.7 ± 0.1	4.2 ± 0.1 (n = 7)
max. BC	23.7 ± 5.3 (n = 6)	34.0 ± 6.1	25.6 ± 3.3	25.0 ± 3.7 (n = 7)
MMR_{ex+dig} (µmol O_2 min^{-1} g^{-1})	0.0853 ± 0.0033 (n = 6)	0.1145 ± 0.0069	0.0994 ± 0.0055	0.1343 ± 0.0039

E_{max} (BL g µmol^{-1})	2431.4 ± 126.3 (n = 6)	2023.0 ± 161.2	2309.5 ± 173.6	1923.3 ± 110.9

[a] U_{crit} value of individuals without burst capacity (n = 1) removed from statistical analysis

Table 2 Summary of test output for SDA (specific dynamic action) response (t-test)

SDA	*0°C – 6°C*		
	t	*df*	*p*
SMR (µmol O_2 min^{-1} g^{-1})	-1.520	8	0.167
MMR_{dig} (µmol O_2 min^{-1} g^{-1})	-3.786	5	0.013*
AS_{dig} (µmol O_2 min^{-1} g^{-1})	-1.699	5	0.150
duration (h)	0.610	5	0.568
magnitude (µmol O_2 g^{-1})	-0.302	5	0.775

* $p < 0.05$

Table 3 Summary of test output for data from exercised specimens (t-test, Mann-Whitney Rank Sum test). Upper part: swimming performance (U_{gait} = gait transition speed, U_{crit} = critical swimming speed, max. BC = maximal burst count); middle part: metabolic performance (SMR = standard metabolic rate, MMR = maximum metabolic rate, AS = aerobic scope); lower part: energetic efficiency of maximum swimming performance (E_{max})

Treatment	*SMR (µmol O_2 min^{-1} g^{-1})*			*MMR (µmol O_2 min^{-1} g^{-1})*			*AS (µmol O_2 min^{-1} g^{-1})*		
	t	*df*	*p*	*t*	*df*	*p*	*t*	*df*	*p*
$0°C_{ex}$ vs. $0°C_{ex+dig}$[a]				-0.695	8	0.507	-0.472	8	0.650
$6°C_{ex}$ vs. $6°C_{ex+dig}$[a]				2.509	14	0.025*	2.361	14	0.033*
$0°C_{ex+dig}$ vs. $3°C/0°C_{ex+dig}$				-3.804	10	0.003**			
$6°C_{ex+dig}$ vs. $3°C/6°C_{ex+dig}$				5.191	14	<0.001***			
$0°C_{ex+dig}$ vs. $6°C_{ex+dig}$[a]				-9.218	12	<0.001***	-5.540	12	<0.001***
$0°C_{ex}$ vs. $6°C_{ex}$	-1.166	13	0.265	-2.241	10	0.049*	-1.887	10	0.088
$3°C/0°C_{ex+dig}$ vs. $3°C/6°C_{ex+dig}$				1.732	12	0.109			

Treatment	*U_{gait} (BL sec^{-1})*			*U_{crit} (BL sec^{-1})*			*max. BC*		
	t	*df*	*p*	*t*	*df*	*p*	*t*	*df*	*p*
$0°C_{ex}$ vs. $0°C_{ex+dig}$	-1.074	7	0.318	3.000[b]		0.167[c]	0.939	8	0.375
$6°C_{ex}$ vs. $6°C_{ex+dig}$	5.076	13	<0.001***	3.781	13	0.002**	12.500[b]		0.072[c]
$0°C_{ex+dig}$ vs. $3°C/0°C_{ex+dig}$	-2.820	10	0.018*	-2.432	10	0.035*	-1.282	10	0.229
$6°C_{ex+dig}$ vs. $3°C/6°C_{ex+dig}$	8.000[b]		0.021[c, *]	3.493	13	0.004**	-0.127	13	0.901
$0°C_{ex+dig}$ vs. $6°C_{ex+dig}$	-7.447	11	<0.001***	-6.286	11	<0.001***	-0.210	11	0.837
$0°C_{ex}$ vs. $6°C_{ex}$	-1.394	9	0.197	-0.673	9	0.518	0.121	10	0.906
$3°C/0°C_{ex+dig}$ vs. $3°C/6°C_{ex+dig}$	0.497	12	0.628	0.350	12	0.733	1.303	12	0.217

Treatment	*E_{max} (BL g µmol^{-1})*		
	t	*df*	*p*
$0°C_{ex}$ vs. $0°C_{ex+dig}$	0.417	8	0.688
$6°C_{ex}$ vs. $6°C_{ex+dig}$	24.000[b]		0.442[c]
$0°C_{ex+dig}$ vs. $3°C/0°C_{ex+dig}$	1.994	10	0.074
$6°C_{ex+dig}$ vs. $3°C/6°C_{ex+dig}$	14.000[b]		0.065[c]
$0°C_{ex+dig}$ vs. $6°C_{ex+dig}$	3.016	12	0.011*
$0°C_{ex}$ vs. $6°C_{ex}$	1.351	10	0.206

3°C/0°C$_{ex+dig}$ vs. 3°C/6°C$_{ex+dig}$	-1.170	12	0.265

[a] AS_{ex+dig} calculated by assigning SMR-values obtained during the unfed state to the respective fed individuals

[b] U-statistic (Mann-Whitney Rank Sum test)

[c] p_{exact}-values obtained by Mann-Whitney Rank Sum test

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 4 Summary of test output for comparison of non-exercised and exercised specimens (t-test, Mann-Whitney Rank Sum test)

Treatment	*MMR (µmol O_2 min^{-1} g^{-1})*			*AS (µmol O_2 min^{-1} g^{-1})*		
	t	*df*	*p*	*t*	*df*	*p*
0°C$_{dig}$ vs. 0°C$_{ex}$	-4.598	6	0.004**	-2.286	6	0.062
6°C$_{dig}$ vs. 6°C$_{ex}$	-0.901	9	0.391	-2.360	9	0.043*
0°C$_{dig}$ vs. 0°C$_{ex+dig}$[a]	-5.031	8	0.001**	-2.212	8	0.058
6°C$_{dig}$ vs. 6°C$_{ex+dig}$[a]	1.000[b]		0.024[c], *	-4.675	9	0.001**

[a] AS_{ex+dig} calculated by assigning SMR-values obtained during the unfed state to the respective fed individuals

[b] U-statistic (Mann-Whitney Rank Sum test)

[c] p_{exact}-values obtained by Mann-Whitney Rank Sum test

* $p < 0.05$, ** $p < 0.01$

4 Discussion

The present thesis addresses impacts of ocean acidification and warming (OAW) on whole-animal metabolism and growth performance of the two co-occurring species Polar cod (*Boreogadus saida*) and Atlantic cod (*Gadus morhua*) in order to predict their future relative competitive strength. In addition to the species comparison at rest, this PhD project aimed to contribute to a more comprehensive picture of acclimation capacities of *B. saida* that attracted distinctly less scientific attention in the past compared to the physiologically well-investigated *G. morhua*. Therefore, maximum performance parameters (swimming and associated respiration) of *B. saida* were investigated under different OAW conditions as well as under food deprivation, considering the decreasing abundance of prey organisms for *B. saida* in a warming Arctic.

In the following, the results of the present PhD project will be discussed comprehensively, while taking into account relevant data from parallel studies that investigated further organizational levels of the very same specimens of both *B. saida* and *G. morhua* (mitochondrial performance: Elettra Leo, behavior: Matthias Schmidt). A summary of temperature-effects for each recorded parameter relative to the respective control conditions is given for *B. saida* and *G. morhua* in TABLE 6 and TABLE 7, respectively.

4.1 Acclimation capacities of resting *B. saida* and *G. morhua* to ocean acidification and warming (OAW) conditions

Standard metabolic rate (SMR) is a crucial parameter for the investigation of species-specific acclimation capacities, because the whole-animal SMR incorporates energetic investment for the oxygen supply system as well as cellular and molecular processes and thereby integrates potential elevated costs under altered abiotic conditions, indicating limits of organismic acclimation capacities (Strobel et al. 2012). By representing inevitable organismic costs at rest, SMR is setting the baseline of energy available for other vital functions. Accordingly, I measured the standard metabolic rate of all specimens involved in the present PhD project.

$\dot{M}O_2$ per unit weight is generally accepted to decrease with increasing fish weight (e.g. Beamish 1964). The body weight of specimens investigated in publication II (25.6 g) was roughly twofold higher compared to publication III (14.0 g). A slightly divergent approach to determine SMR was further expected to intensify a discrepancy between relative $\dot{M}O_2$

values in bigger fish of publication II (15 % quantile of $\dot{M}O_2$ data) and smaller fish of publication III (20 % quantile of $\dot{M}O_2$ data). Nevertheless, the SMR of *B. saida* investigated in publication II was approximately one and a half times higher than in publication III at both 0 and 6°C (when applying the same mass correction factor, thereby ruling out size effects). This contradictory observation is likely attributed to a distinctly shorter food deprivation period of *B. saida* in publication II (4 days compared to 18 – 21 and 10 – 13 days at 0 and 6°C, respectively in publication III). Investigations of the SDA response in this species revealed a duration of postprandially elevated $\dot{M}O_2$ of 167 h (0°C) and 135 h (6°C) (19 % decrease in SDA duration between 0 and 6°C) (publication III). Hop and Tonn (1998) even documented gastric evacuation half-times of 146 h for *B. saida* at -0.49°C. Accordingly, the SMR of *B. saida* investigated in publication II is most likely still influenced by a remittent SDA signal that represents the costs for the assimilation and transformation of nutrients (e.g. Jobling 1981, Brown and Cameron 1991a, b). Similar SMR values in publication II and *B. saida* from the Canadian Arctic at comparable temperatures recorded two days after feeding (Drost et al. 2016) also imply that an elevated gastrointestinal oxygen demand adds onto true metabolic baseline costs in the fish of the latter study.

The SMR of *B. saida* (publication I – III) and *G. morhua* (publication I) following long-term acclimation to elevated temperatures with 8 and 16°C representing the respective highest acclimation temperatures in my studies, is a fundamental component for the investigation of species-specific thermal tolerance limits in the present study. In general, warm-acclimation involves molecular and cellular adjustments of functional capacities such as changes in membrane composition towards a more rigid structure (homeoviscous adaptation) (e.g. Hazel 1988) and associated adjustments in enzyme and mitochondria capacities, resulting in a reduction of thermally-induced elevated maintenance costs (Pörtner et al. 2017). The SMR of *B. saida* acclimated for four months (publication I & II) and 1.5 months (publication III) to up to 6°C was comparable to control conditions at 0°C at both normocapnic (390 µatm) and hypercapnic (1170 µatm) water conditions (TABLE 6). *B. saida* acclimated to 8°C, however, did not show full metabolic compensation (compare type II compensation, Precht 1958), visible in a significantly elevated SMR, pointing towards *B. saida*'s long-term upper thermal tolerance limits (pejus temperatures (T_{pej}) sensu Pörtner et al. 2017). In fact, the polar stenothermal *B. saida* revealed limited capacities to adjust enzyme activities in cardiac mitochondria after long-term acclimation to elevated

temperatures (Leo et al. in prep.). Further, plasmatic membrane lipid class compositions showed few adjustments in response to warm-acclimation (Leo et al. in prep.), potentially resulting in a significantly elevated mitochondrial proton leak and associated reduced ATP production efficiencies of specimens acclimated to 8°C (Leo et al. 2017), in part explaining the elevated whole-animal SMR compared to control conditions at 0°C.

Compared to *B. saida*, *G. morhua* revealed higher capacities to adjust both enzyme activities and membrane composition to elevated acclimation temperatures (Leo et al. in prep.). Nevertheless, acclimation processes could not fully counteract thermally-induced rising metabolic costs in *G. morhua* (compare type III – V compensation, Precht 1958), resulting in an increasing whole-animal SMR of *G. morhua* within the range of experimental temperatures (3 – 16°C) at both PCO_2 conditions (publication I) (TABLE 7). Increasing muscular activity (quantified in terms of spontaneous activity) with temperature holding the potential to interfere with SMR as proposed by Green und Fisher (2004), however, was not detected for *G. morhua* in the present study (Schmidt et al. 2017).

A comparison of SMR between *B. saida* and *G. morhua* at 3 and 8°C revealed significantly higher metabolic baseline costs of *B. saida* at both acclimation temperatures, indicating higher mitochondrial densities or enhanced rates of mitochondrial substrate oxidation (Guderley 2004) in the Arctic endemic *B. saida*. Indeed, mitochondrial respiration was elevated in *B. saida* compared to *G. morhua* at both 3 and 8°C (Leo et al. 2017). Accordingly, under comparable environmental temperatures, *B. saida* needs to invest a higher energetic fraction for baseline metabolism with potential trade-offs for fitness-related functional capacities. Nevertheless, in order to fully evaluate a potential fitness-related disadvantage for *B. saida* relative to *G. morhua*, investigations of the response of MMR, and thereby the AS, of both species are necessary. However, maximum performance parameters of *G. morhua* were not recorded during the present PhD project due to major drawbacks during swim tunnel experiments (see paragraph 4.3.1).

TABLE 6: Temperature-effects for parameters of *B. saida* within PCO_2- and feeding levels listed relative to the respective control conditions (0°C/390 µatm, 0°C/1170 µatm, 0°C/unfed and 0°C/fed, respectively). The treatment 6°C/3°C is considered relative to 6°C/fed. Colors illustrate if a parameter increases (green) or decreases (red).

BOREOGADUS SAIDA								
Routine parameters	**390 µatm CO_2**				**1170 µatm CO_2**			
	0°C	**3°C**	**6°C**	**8°C**	**0°C**	**3°C**	**6°C**	**8°C**
SGR		-	(↑)	-		-	-	-
F*		(↑)	↑	(↑)		↑	↑	↑↑↑
FCE*		(↓)	-	↓↓↓		(↓)	(↓)	↓
CF*		-	-	↓		(↑)	(↓)	-
HSI		-	-	-		-	-	-
GSI male*		(↓)	-	↓		-	(↓)	↓
SMR*		-	-	↑↑		-	(↑)	↑
Maximum parameters	**390 µatm CO_2**				**1170 µatm CO_2**			
	0°C	**3°C**	**6°C**	**8°C**	**0°C**	**3°C**	**6°C**	**8°C**
MMR		-	(↑)	(↑)		(↑)	↑	↑↑
AS		-	-	-		(↑)	↑	(↑)
U_{gait}		-	-	(↑)		-	-	(↑)
U_{crit}		-	(↓)	-		-	-	-
E_{max}		-	(↓)	(↓)		↓	↓	↓
BC_{max}		-	-	-		-	-	-
BC_{tot}		-	(↓)	-		-	-	(↓)
TSB		-	(↓)	-		-	-	-
$TSB_{anaerob}$		(↑)	-	-		(↑)	-	-
Digestion versus exercise	**0°C**		**unfed 6°C**		**0°C**	**0°C/3°C**	**fed 6°C/3°C**	**6°C**
SMR			-					
MMR_{ex} & MMR_{ex+dig}			↑			↑↑	↓↓↓	↑↑↑**
MMR_{dig}								↑
AS_{ex} & AS_{ex+dig}			(↑)					↑↑↑
AS_{dig}								-
SDA duration								-
SDA magnitude								(↑)
U_{gait}			-			↑	↓	↑↑↑**
U_{crit}			-			↑	↓↓	↑↑↑**
E_{max}			-			(↓)	(↑)	↓
BC_{max}			-			(↑)	-	-

routine parameters: *post hoc* Tukey honest significance test following one-way ANOVA or max-t test; maximum parameters: *post hoc* Tukey honest significance test following two-way ANOVA; digestion versus exercise: t-test or Mann-Whitney Rank Sum test; -: no temperature effect; arrows in brackets: non-significant trends, single arrows: $p < 0.05$, double arrows: $p < 0.01$, triple arrows: $p < 0.001$; * max-t test; ** significant difference between fed and unfed treatment.

TABLE 7: Temperature-effects for parameters of *G. morhua* within PCO_2-levels listed relative to the respective control conditions (0°C/390 µatm, 0°C/1170 µatm). Green color indicates increasing parameters.

GADUS MORHUA								
Routine	**390 µatm CO_2**				**1170 µatm CO_2**			
parameters	**3°C**	**8°C**	**12°C**	**16°C**	**3°C**	**8°C**	**12°C**	**16°C**
SGR		↑↑↑	↑↑↑	↑↑↑		↑↑	↑↑↑	↑↑↑
F*		↑↑	↑↑↑	↑↑↑		↑	↑↑↑	↑↑
FCE*		↑	(↑)	(↑)		↑	↑	↑
CF*		(↑)	(↑)	↑		(↑)	-	↑↑
HSI		-	-	-		↑	↑↑↑	↑↑↑**
GSI male*		-	-	-		-	-	-
SMR*		↑↑	↑↑↑	↑↑↑		↑↑↑	↑↑↑	↑↑↑

routine parameters: *post hoc* Tukey honest significance test following one-way ANOVA or max-t test; -: no temperature effect; arrows in brackets: non-significant trends, single arrows: $p < 0.05$, double arrows: $p < 0.01$, triple arrows: $p < 0.001$; * max-t test; ** significant PCO_2-effect.

In line with previous studies on *G. morhua* (Melzner et al. 2009a, Kreiss et al. 2015), the whole-animal SMR of both gadoid species was not affected by elevated PCO_2 conditions. Nonetheless, the SMR of *B. saida* at 0 and 8°C as well as the SMR of *G. morhua* at all temperatures except 12°C showed a trend to be non-significantly reduced under hypercapnia. Likewise, the spiny damselfish (*Acanthochromis polyacanthus*) revealed reduced baseline metabolic costs after acclimation to 950 µatm (Rummer et al. 2013b). $\dot{M}O_2$ measurements are known to be affected by spontaneous activity with a magnitude that is difficult to quantify (Chabot et al. 2016a). Investigations on spontaneous activity in specimens of the present study, however, did not reveal any clear impact of PCO_2 levels (Schmidt et al. 2017). When keeping in mind that highly dosed CO_2 is used as an anesthetic for fish (e.g. Gelwicks et al. 1998), a potential sedative effect of near-future PCO_2 levels, therefore, is unlikely to explain the trends observed for SMR of *B. saida* and *G. morhua*.

4.2 Growth performance of *B. saida* and *G. morhua* under OAW conditions

Somatic growth performance is directly related to individual fitness and survival, thereby mediating species abundance. Accordingly, growth performance measurements under elevated temperatures and PCO_2 conditions are of utmost importance for the investigation of the respective species' future competitive strength.

The specific growth rate (SGR) of *B. saida* from Svalbard waters detected in the present study (0°C: 0.386 % d^{-1}; 3°C: 0.449 % d^{-1}; 6°C: 0.509 % d^{-1}; 8°C: 0.349 % d^{-1}) (publication I) was in line with the growth performance of *B. saida* originating from the Pechora Sea (0°C: ~ 0.36 % d^{-1}) (Christiansen 1995). However, the SGR in both studies was distinctly lower than of *B. saida* from the Beaufort Sea of similar size (0°C: 0.729 % d^{-1}; 5°C: 1.348 % d^{-1}; 9°C: 1.387 % d^{-1}; 16°C: -0.152 % d^{-1}) (Laurel et al. 2015) at comparable temperatures. The lower growth performance in my study and the study of Christiansen (1995) is likely attributed to less frequent feeding events: While I and Christiansen (1995) fed the fish every fourth day in order to simulate projected decreases in prey availability for *B. saida* due to a progressive borealisation of prey organisms (Fossheim et al. 2015), Laurel et al. (2015) applied a daily feeding schedule. This may have attenuated thermal effects on the growth performance of *B. saida* within both PCO_2 levels in my study (TABLE 6). Nevertheless, I observed a non-significant trend for optimum growth performance of *B. saida* at 6°C under control PCO_2 conditions, 1.3°C below the thermal optimum detected by Laurel et al. (2015). The optimum temperature for growth (T_{opt}) is known to shift to lower temperatures during less favorable feeding conditions (Brett et al. 1969, Jobling 1994). Wild fish, therefore, are commonly more abundant at temperatures below their T_{opt} obtained during *ad libitum* food conditions in the laboratory (Björnsson et al. 2001). Accordingly, a growth optimum at 7.3°C (Laurel et al. 2015) likely exceeds the T_{opt} of *B. saida* in the field.

Despite reduced growth performance, *B. saida* is well-adapted to temperatures close to the freezing point, visible in a significantly elevated food conversion efficiency (FCE) at 0°C indicating well-functioning digestion at low temperatures as observed by Hop et al. (1997). The low growth performance of *B. saida* at 0°C, therefore, is likely attributed to a reduced voluntary food intake in the cold detected in publication I and III. At thermal conditions below the T_{opt}, food intake is restricted due to slow stomach evacuation rates (Hop and Tonn 1998) (see paragraph 4.1). Prolonged gastric retention times, however, result in high assimilation rates (0°C: 80 %, Hop et al. 1997). In combination with a reduced SDA magnitude (in response to a lower voluntary food intake compared to 6°C-acclimated *B. saida*) (publication III), which represents the energetic expenditure for digestion, and low baseline metabolic costs (publication I – III), high energetic fractions remain for the growth performance of *B. saida* at 0°C.

The cold-adaptation of *B. saida* is further expressed in a (non-significantly) elevated gonadosomatic index (GSI) at 0°C. A statistically significant difference of GSI between temperature treatments, however, was disguised by a high standard deviation that decreased with increasing acclimation temperature indicating a decreasing percentage of maturing individuals with rising temperature. Decreasing GSIs with acclimation temperature entail a reverse trend for the hepatosomatic index (HSI) (publication I) (TABLE 6) similar to observations by Nahrgang et al. (2014), because liver energy stores are depleted during gonad development (Hop et al. 1995). Gonads of *B. saida* begin to develop in August, with a more rapid development reported for males compared to female congeners (Hop et al. 1995). The majority of specimens involved in the present study were male (85.2 %), revealing GSI values at 0°C (7.3 %) (publication I) comparable to those measured for male specimens from the Canadian Arctic at 1°C in September (~ 8 %) (Hop et al. 1995), while females showed a GSI of only 2.1 % (publication I). Despite favorable conditions for *B. saida* at 0°C implied by the enhanced GSI, one might argue that the energetic investment in gonad development may have contributed to the low growth performance observed at this temperature. However, Christiansen (1995) did not observe differences in growth performance between sexes before the winter months (September – January), when males reached peak GSIs of 30 % (Hop et al. 1995). Accordingly, if any, a depression in growth performance evoked by energetic cost for gonad development is neglectable for the time period considered in this thesis.

In the field, *B. saida* has been reported to prefer a narrow thermal range (1 – 2°C) during spawning (Hognestad 1966). My results indicate that rising water temperatures at spawning grounds may induce delayed spawning periods resulting in a potential temporal mismatch with the peak appearance of suitable prey organisms as projected for various Arctic species (Larsen et al. 2014) due to species-specific thermal sensitivities. Moreover, warmer temperatures might cause lower energetic investment in gonad development, resulting in reduced reproductive output of *B. saida* as observed in fjords at the westcoast of Svalbard that are highly influenced by Atlantic water masses (Nahrgang et al. 2014) with likely cascading consequences for the Arctic food web, providing that a shift of the spawning areas to sufficiently colder waters is not possible. Especially with an ongoing decrease in sea ice, which represents a typical spawning habitat for this species (Rass 1968), alternative spawning grounds are expected to become sparse.

At 8°C, the growth performance of *B. saida* was suboptimal, attributed to elevated standard metabolism (publication I & II) which, in turn, was evoked by mitochondrial limitations (Leo et al. 2017) as discussed above (paragraph 4.1). Although Drost et al. (2014) reported a thermal habitat range of *B. saida* from the Canadian Arctic of up to 8°C, mortality, exclusively occurring at 8°C towards the end of the four months lasting growth experiment (control CO_2 treatment: n = 1, high CO_2 treatment: n = 2, publication I), indicates that *B. saida* can encounter this temperature only for limited time periods. Consequently, 8°C must be considered to be already beyond the long-term upper T_{pej} of juvenile *B. saida*.

An onset of organismic limitations of *B. saida*, however, could already be observed at acclimation temperatures above 3°C indicated by a decreasing condition factor (CF) and a non-increasing food intake between 3 and 8°C (publication I). Commonly, appetite and therefore the amount of voluntary food consumption are triggered by accelerated biochemical processes with increasing temperature as well as increasing intestinal peristalsis that result in shortened gastric retention times (Jobling and Spencer Davies 1979) and gut passage rates (Beamish 1974). The possibility that the (stable) amount of consumed food above 3°C represented the maximum consumable volume, thereby indicating total stomach emptying between feeding events, was excluded by stomach content investigations in *B. saida* in the end of the study.

In contrast to *B. saida*, the growth performance of *G. morhua* was positively correlated with acclimation temperature (3 – 16°C) with the increase in growth being less pronounced above 8°C (control CO_2 treatments) and 12°C (high CO_2 treatments). Accordingly, no apparent T_{opt} could be determined in the present study (publication I). Björnsson et al. (2007), however, reported a T_{opt} of 12.1°C for *G. morhua* from Icelandic waters of similar size (56.9 g) compared to specimens in the present study (43.4 g). Early juveniles (56 days) from the same population as *G. morhua* used in the present growth experiment (NEAC) showed a T_{opt} of 14.2°C (Otterlei et al. 1999). Considering that T_{opt} decreases between populations with increasing latitudinal origin (Fischer 2002) as well as with increasing body size (Björnsson et al. 2007), it is surprising that T_{opt} does not seem to be covered by the range of experimental temperatures investigated in my study. Drost et al. (2016) presented evidence that acclimation experiments neglecting the impact of activity might result in an overestimation of thermal capacities: While their *B. saida* acclimated to 6.5°C

revealed the highest aerobic scope, this acclimation group showed distinct mortality rates (50 %) after exercise under acute exposure to 8.5°C. In my growth experiment, *G. morhua* were kept in individual tanks in order to prevent cannibalism and to determine the exact amount of individually consumed food. Individual housing in comparably small tanks (~ 24 L) caused the radius of movement to be limited in addition to a lack of feeding competition. As a result, spontaneous activity of *G. morhua* was unaffected by temperature (Schmidt et al. 2017), although activity is commonly known to increase in the warmth (Brown et al. 1989). Depressed activity in the present growth study was further indicated by a mortality of 100 % a few hours after exercise experiments at 12 and 16°C (not performed at 3 and 8°C) (see paragraph 4.3.1). This supports the assumption, that energetic reallocation in favor of growth may have increasingly taken place at higher temperatures, thereby potentially masking T_{opt} of *G. morhua* in the present study.

Yet, I recorded distinctly lower growth rates at 12°C (0.78 % d^{-1}) (publication I) compared to Islandic *G. morhua* at 12.1°C (1.75 % d^{-1}) (Björnsson et al. 2007), most likely caused by the low feeding frequency established in my study (every fourth day), while Björnsson et al. (2007) fed their fish 3 – 5 times per day. Accordingly, when applying temperature-dependent weight data of specimens of the present study (incubation midterm) to a maximum capacity growth model for *G. morhua* (Butzin and Pörtner 2016), a potential for significantly higher growth performance was detected (FIG. 18). A compensation of low feeding frequencies through large meal sizes was not detected, indicated by a distinctly lower daily food uptake detected in my fish (0.81, 0.98 and 1.16 % BW d^{-1} at 8, 12 and 16°C, respectively) compared to similar-sized *G. morhua* fed every second day at comparable acclimation temperatures (3.67, 3.73 and 5.14 % BW d^{-1} at 7, 10 and 15°C, respectively) (Soofiani and Hawkins 1982). Accordingly, I recorded total stomach emptying at all temperatures above 3°C. Hence, an apparent food-restriction prevented *G. morhua* from expressing their full growth capacity. Positive growth throughout all treatments (exception: n = 1 at 3°C) as well as the increasing CF with temperature (publication I) (TABLE 7), however, indicate that food consumption was sufficient to cover rising metabolic costs, thereby excluding the possibility of starvation.

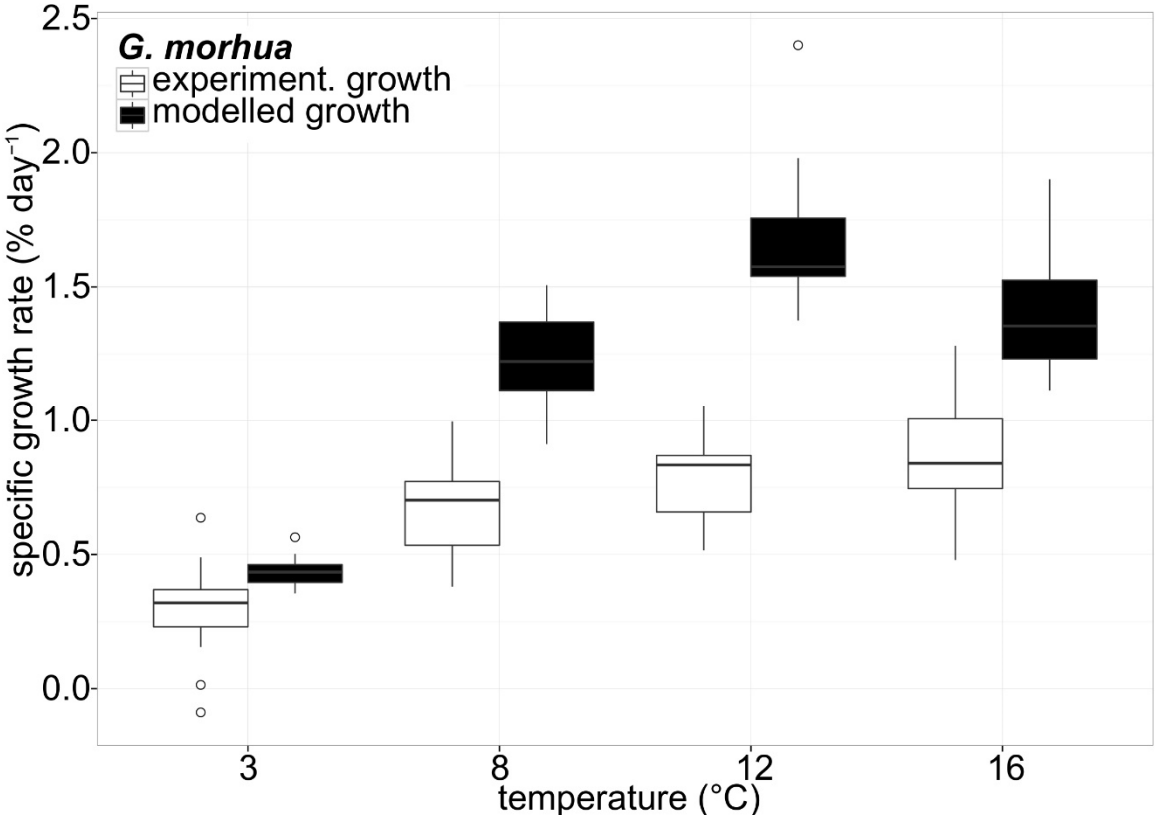

FIGURE 18. Comparison of experimental growth data (mean weight during the time span of the growth experiment) (publication I) and modelled growth performance (Butzin and Pörtner 2016) for *G. morhua*.

While all recorded routine parameters consistently suggest an enhanced performance of *G. morhua* with rising acclimation temperature, the significantly lowered FCE at the coldest acclimation temperature (3°C) indicates cold-induced limitations of the digestive system thereby suggesting 3°C to represent the lower T_{pej} of this population. Nevertheless, Michalsen et al. (2014) observed NEAC to occasionally even experience subzero temperatures, presumingly triggered by a higher abundance and higher energy content of prey organisms.

The growth performance of the Arctic endemic *B. saida* and the cold-temperate population of *G. morhua* was comparable at 3°C, while the growth performance of *G. morhua* significantly exceeded the one detected for *B. saida* at 8°C. A similar relation of growth performance was observed for *B. saida* originating from the Beaufort Sea and a cold-adapted Pacific gadoid (Pacific cod, *Gadus microcephalus*) (Laurel et al. 2015). It has to be mentioned, however, that both size-at-age data (Falk-Petersen et al. 1986) and gonad

inspections suggest *B. saida* specimens to be older (approximately age 2) than *G. morhua* (age 1) in the present study. Accordingly, conclusions drawn from a direct comparison of growth performance of both species have to be considered cautiously due to lower growth rates of older individuals, especially when keeping in mind the distinctly lower maximum body size of *B. saida* compared to *G. morhua*.

Despite a lower thermal sensitivity compared to true stenothermal Antarctic fish species (e.g. Brodte et al. 2006), temperature-dependent growth performance recorded in the present study clearly indicates a rather stenothermal lifestyle of the high latidude species *B. saida* compared to the cold-eurythermal *G. morhua*. Accordingly, although both species are inhabiting waters at the lower end of their thermal range (*B. saida*: -1.7 – 2.0°C in bottom waters, TABLE 1, -1.8 – -1.1°C in under-ice habitats, David et al. 2015; *G. morhua*: 4.0 – 6.0°C, TABLE 1), the present results suggest future water temperatures to promote the performance of the eurythermal *G. morhua*. Besides direct effects of rising water temperatures, the progressive invasion of *G. morhua* into Svalbard waters (Berge et al. 2015) entails an increasing predation pressure on *B. saida*. While deep water layers are expected to experience a less pronounced increase in temperature compared to surface waters (Pavlov et al. 2013), already a small temperature rise in deep water habitats of *B. saida* may be sufficient to prolong occasional forage excursions of *G. morhua*, thereby further decimating the stock size of the already physiologically limited *B. saida*.

Compared to thermal effects on the resting performance of *B. saida* and *G. morhua*, effects of elevated PCO_2 conditions were more subtle. In contrast to the situation under normocapnia, the growth performance of *B. saida* under high PCO_2 conditions was not affected by acclimation temperature, thereby not expressing a T_{opt}. The growth performance of *G. morhua*, however, was unaffected by high PCO_2 levels. In line with findings for *B. saida*, Miller et al. (2012) found a depressed growth performance in juvenile Cinnamon anemonefish (*Amphiprion melanopus*) exposed for 31 days to 1030 µatm CO_2. While Miller et al. (2012) could attribute this result to elevated baseline metabolic costs, the SMR of *B. saida* was not affected by 1170 µatm PCO_2 in the present study (see paragraph 4.1), thereby not serving as an explanation for the observed impairment in growth performance. A higher sensitivity in the growth performance of *B. saida* to future OA levels may be evoked by a more stable habitat concerning PCO_2 levels compared to

fluctuations experienced by the highly active *G. morhua* (Neuenfeldt et al. 2009). Accordingly, Moran and Støttrup (2011) first detected impaired growth performance of juvenile *G. morhua* under PCO_2 conditions as high as 8500 µatm. Previous exposure of parental fish to the same elevated PCO_2 level, however, reversed negative effects detected on growth performance (Miller et al. 2012). Accordingly, it remains to reveal, whether (and to which degree) *B. saida* is able to mitigate effects evoked by hypercapnia through transgenerational adaptation.

The most distinct PCO_2-effect on resting parameters was detected in a significantly elevated HSI of *G. morhua* at 16°C. Likewise, Kreiss et al. (in prep.) detected an elevated HSI under hypercapnic conditions (2200 µatm) at 18°C that was not apparent after four weeks of acclimation to 10°C. In the same study, elevated liver enzyme capacities (namely phosphoenolpyruvate carboxykinase) were recorded that indicate an enhanced storage of carbohydrates, potentially causing the observed increase in HSI (Kreiss et al. in prep.). A potential shift from lipid to carbohydrate metabolism has previously been interpreted as a warm-acclimation strategy in Antarctic fish close to species-specific thermal extremes as carbohydrates serve as efficient energy reserves under thermally-induced reduced oxygen conditions (Windisch et al. 2011). Indications for this so-called warm-hardiness under high PCO_2 conditions in my study and the study of Kreiss et al. (in prep.) suggest onsetting heat tolerance limitations (Windisch et al. 2011) of *G. morhua* at 16°C that were not visible under control PCO_2 levels.

4.3 Maximum performance parameters of *B. saida* under future water conditions

The maximum metabolic rate (MMR) represents an organism's maximum capacity of oxygen transport to tissue, thereby determining the ceiling of the aerobic scope (AS). By representing aerobic energy production and usage capacities at the respective environmental conditions, the AS sets the margins for all aerobic processes exceeding baseline metabolism such as growth, digestion and swimming performance (Fry and Hart 1948, Brett 1964, Fry 1971). Accordingly, in order to assess climate-induced habitat limitations of single populations, knowledge about long-term effects of elevated temperatures and PCO_2 conditions as well as food deprivation on the AS are essential.

Traditionally, MMR is quantified as the maximum possible $\dot{M}O_2$ following intense exercise (Muir and Niimi 1972). Swimming capacity plays an important role during spawning migrations in some species (maximum sustainable swimming capacity, here represented by U_{gait}) as well as during foraging, escape reactions in response to predator encounters and strong currents (critical swimming speed, U_{crit} and burst-and-coast swimming performance). Accordingly, both endurance and sprint performance capacities are hypothesized to relate to individual fitness and population abundance (Beamish 1978). The ecological relevance of both aerobic sustained and anaerobic swimming capacities, however, differs based on species-specific behavioral strategies (Dunn and Johnston 1986, Clark et al. 2013).

In the following, maximum swimming capacity and the associated MMR (and thereby AS) of *B. saida* under OAW conditions as well as food deprivation scenarios will be discussed first (paragraph 4.3.1) before the focus is directed to the anaerobic swimming capacity of *B. saida* and its potential ecological relevance (paragraph 4.3.2).

4.3.1 Maximum aerobic performance

In addition to the SMR, I recorded the maximum metabolic rate of unfed *B. saida* evoked by exhaustive exercise (MMR_{ex}) in order to investigate the AS of *B. saida* under different abiotic (publication II & III) and biotic (publication III) conditions. Maximum performance parameters of *G. morhua*, however, were not recorded, because none of the investigated individuals of this species (n = 10) did recover from exhaustive exercise (U_{crit} protocol). Previous studies, in turn, did not report mortality of *G. morhua* following U_{crit} protocols (e.g. Bushnell et al. 1994, Melzner et al. 2009a). Therefore, I hypothesize that limited activity in response to small housing during the growth period may have resulted in insufficiently developed cardiopulmonary capacities to match the high oxygen demand of the red swimming muscle. Wood et al. (1983) excluded heart failure, instead suggesting an intracellular acidosis to cause mortality of rainbow trout (*Oncorhynchus mykiss*) following intense exercise.

The MMR_{ex} of *B. saida* measured in publication II was similar to the MMR_{ex} detected for *B. saida* originating from the Canadian Arctic at comparable acclimation temperatures (Drost et al. 2016). Nevertheless, the MMR_{ex} obtained in publication II (0°C: 0.0993 µmol O_2 min^{-1} g^{-1}, 6°C: 0.1259 µmol O_2 min^{-1} g^{-1}) was significantly higher than in publication

III (0°C: 0.0739 µmol O_2 min^{-1} g^{-1}, 6°C: 0.1033 µmol O_2 min^{-1} g^{-1}), when adjusting $\dot{M}O_2$ values to a common average fish weight (22.7 g). This difference is likely attributed to different approaches to determine maximum $\dot{M}O_2$ (see paragraph 2.5.5.1).

The MMR_{ex} of *B. saida* rose with acclimation temperature (publication II & III) before it levelled off at temperatures above 6°C, indicating incipient limitations in the oxygen supply capacity (Pörtner 2010). The positive correlation of acclimation temperature and MMR_{ex} is likely evoked by increasing rates of biochemical reactions with temperature (Seebacher et al. 2005). Nevertheless, (non-significantly) reduced MMR_{ex+dig} values (maximum metabolic rate evoked by exercise in fed fish) of 6°C acclimated compared to 0°C acclimated *B. saida* acutely exposed to 3°C (publication III) suggest that compensation mechanisms (although not full compensation) are also acting on the maximum $\dot{M}O_2$ of *B. saida* acclimated up to 6°C (with 6°C representing the highest investigated acclimation temperature). Likewise, Drost et al. (2016) found a lower MMR_{ex} in 6.5°C compared to 1.0°C acclimated *B. saida* when acutely measured at 3.5°C likely due to compensated maximum heart rates in response to warm-acclimation (~ 32 bpm and ~ 27 bpm in cold and warm-acclimated fish, respectively, at a common intermediate temperature).

The increasing MMR_{ex} with acclimation temperature (publication II & III, Drost et al. 2016), combined with the higher SMR at 8°C, resulted in a peak aerobic scope for exercise (AS_{ex}) at 6°C (publication II). The peak AS_{ex} at 6°C coincides with the T_{opt} for growth of *B. saida* in a laboratory environment excluding feeding competition (due to individual housing) and reducing foraging effort (publication I). In the field, non-optimum prey availability, however, most likely demands a higher foraging activity potentially interfering with the energy available for growth performance. Accordingly, despite maximized aerobic capacities at 6°C, *B. saida* is commonly distributed in habitats characterized by temperatures around the freezing point, indicating that maximum exploitation of AS is not a precondition for survival (Deutsch et al. 2015) (see paragraph 4.2).

Along similar lines of reasoning, the peak AS_{ex} at 6°C did not translate into an elevated swimming performance. Instead, statistical analysis revealed a moderate increase in U_{gait} of *B. saida* with acclimation temperature (publication II). This increase mainly refers to an elevated value at the highest investigated temperature (8°C), while visual inspection of U_{gait} data of fish acclimated to 0 – 6°C indicates no apparent temperature-effect as verified by the results obtained for publication III. For U_{crit}, no correlation with acclimation

temperature was observed (publication II & III), similar to results obtained for the Antarctic stenotherm bald notothen (*Pagothenia borchgrevinki*) that did not show any reduction in U_{crit}, even at acclimation temperatures exceeding its habitat temperature by about 4.5°C (Seebacher et al. 2005). Hence, *B. saida* has the capacity to retain its swimming performance throughout the range of investigated acclimation temperatures. The concomitantly increasing MMR_{ex} in the warmth, however, suggests a reduction in the maximum swimming efficiency (E_{max}) at acclimation temperatures > 3°C (normocapnic treatments) (publication II & III), coinciding with the temperature that marks an incipient reduction in the condition factor (CF) (see paragraph 4.1) (publication I). A decreasing E_{max} likely reflects the limited adjustment of plasmatic membrane compositions (Leo et al. in prep.) entailing reduced ATP production efficiencies (Leo et al. 2017) (see paragraph 4.1). In addition, the swimming performance (both U_{gait} and U_{crit}) of *B. saida* acutely exposed to an intermediate temperature (3°C) was comparable in both cold- and warm-acclimated groups, also indicating limited acclimation capacities of the oxidative skeletal muscle after four months of warm-acclimation (publication III).

So far, I can summarize that warm-acclimation resulted in a moderate compensation of the MMR_{ex} and in a maintenance of the swimming performance of *B. saida* throughout a broad thermal range, indicating that aerobic swimming capacity may be a fitness parameter of high ecological relevance for this species. This seems surprising considering the rather sluggish behavior observed for specimens in under-ice habitats (Lønne and Gulliksen 1989, Gradinger and Bluhm 2004). Nevertheless, two major spawning grounds are identified for *B. saida* in the Barents Sea based on local larvae aggregations during late summer: one along the east coast of Svalbard and one in the eastern Barents Sea at the southwest coast of Novaya Zemlya (Gjøsæter 2009). Although there is an inceptive debate, whether there are specific fjord and oceanic populations of *B. saida* similar as described for *G. morhua* (Madsen et al. 2015), spawning migrations might be an explanation for *B. saida* to have evolved an effective aerobic swimming performance little affected by thermal habitat conditions. However, a reduction in E_{max} at acclimation temperatures above 3°C indicates beginning performance limitations, similar to observations on resting parameters (see paragraph 4.2).

In contrast to observations in resting parameters (see paragraph 4.1 and 4.2), near-future PCO_2 levels had a strong impact on the maximum performance of *B. saida*: Similar to results obtained from the tropical reef fish *A. polyacanthus* (17 days at 950 µatm, Rummer et al. 2013b) and the temperate European sea bass (*Dicentrarchus labrax*) (1.5 years at 1500 µatm, A. Crespel et al. pers. communication), the MMR_{ex} of *B. saida* was significantly elevated after four months of exposure to 1170 µatm at all acclimation temperatures higher than 0°C (publication II). In combination with the SMR being unaffected, the AS_{ex} of *B. saida*, thus, was higher in hypercapnic compared to normocapnic treatments (FIG. 19). Interestingly, *B. saida* still showed a significantly reduced maximum swimming performance under high PCO_2 conditions (both U_{gait} and U_{crit}) and hence a lower E_{max} between 3 and 8°C in comparison to normocapnic treatments (publication II). At control PCO_2 levels, the decrease in E_{max} first appeared at warmer acclimation temperatures (6°C), implying higher thermal sensitivities of maximum performance parameters of *B. saida* under hypercapnic conditions.

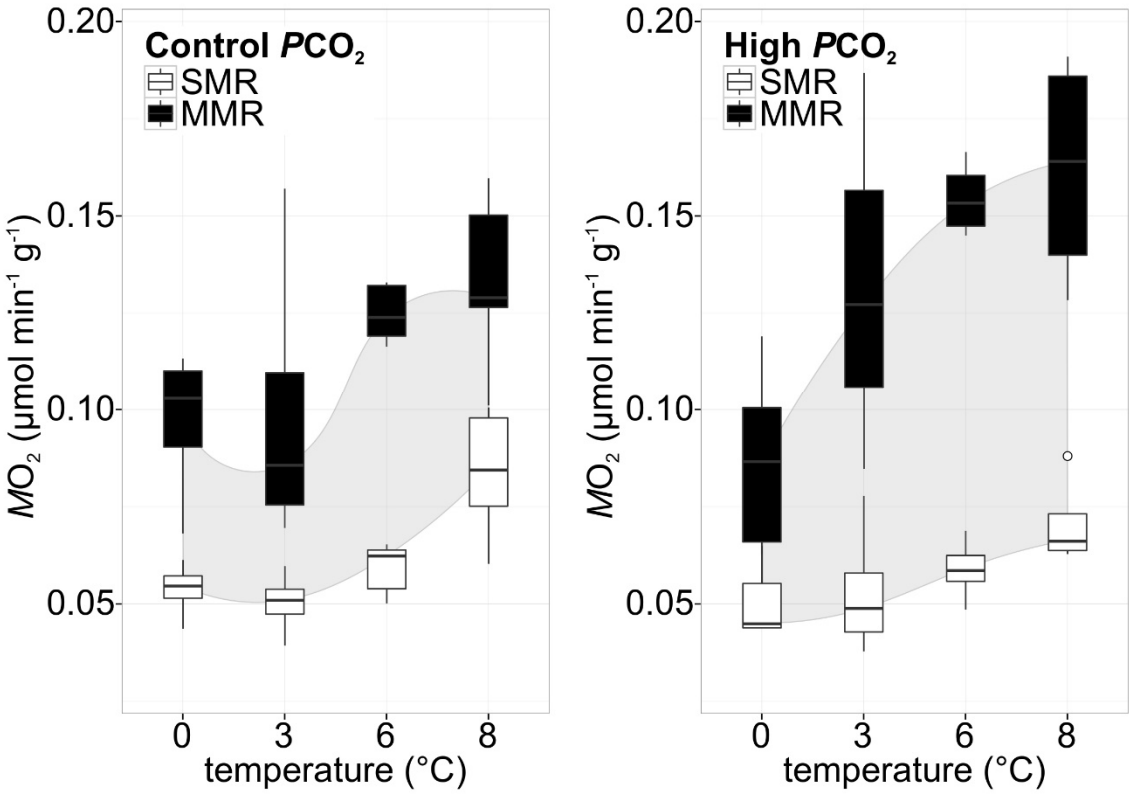

FIGURE 19. Standard metabolic rate (SMR) (white bars) and maximum metabolic rate evoked by exercise (MMR_{ex}) (black bars) of *B. saida* under control (390 µatm) (left) and high PCO_2 (1170 µatm) conditions (right) (publication II). The shaded area depicts the aerobic scope for exercise (AS_{ex}) at the respective PCO_2 levels.

The so far only available study reporting elevated MMR_{ex} under high PCO_2 levels (Rummer et al. 2013b) raised a hypothesis to explain this finding, which is briefly presented in the following, despite limited applicability for *B. saida*. In the following, this hypothesis is critically discussed in the frame of the results of the present study.

Rummer et al. (2013b) speculated that an elevated MMR_{ex} in *A. polyacanthus* after acclimation to OA scenarios (940 µatm) may be evoked by the release of catecholamines triggered by an extracellular acidosis in response to exercise combined with a mild environmental acidosis, thereby facilitating oxygen uptake at the gills and potentially oxygen delivery to the tissue. An enhanced oxygen delivery under high PCO_2 conditions presumes the existence of plasma-accessible carbonic anhydrase (CA) in highly oxygen demanding tissue such as muscle capillaries causing a local acidification and thereby a short-circuiting of the catecholamine-mediated pH stabilization of red blood cells as recently discovered in salmonids (Rummer and Brauner 2011, Rummer et al. 2013a).

Despite a lack of data in the present thesis for a closer investigation of this hypothesis, this hypothesis seems to have little applicability for *B. saida* out of three major reasons: i) *B. saida* has not been found to express elevated catecholamine levels (neither adrenalin nor noradrenalin) following exhaustive exercise (chasing for 5 min) (Whiteley et al. 2006) similar to the cholinergic activity stress response of Antarctic fish (Egginton 1997). ii) An enhanced oxygen delivery to the red swimming muscle under high PCO_2 conditions as suggested by Rummer et al. (2013b) implies an improved swimming performance. *B. saida*, however, displayed a depressed U_{gait} and U_{crit} despite elevated MMR_{ex} indicating elevated costs due to aerobic processes other than swimming. iii) The mechanism of plasma accessible CA-mediated short-circuiting of red blood cell pH_i proposed to facilitate erythrocytic oxygen unload in red muscle tissue has so far only been observed in salmonids (*O. mykiss*, Rummer et al. 2013a) characterized by a highly active lifestyle. Its existence, however, – despite not been investigated – is highly questionable in the little active *B. saida*.

A more likely, albeit still hypothetical approach to explain a reduced swimming performance despite elevated MMR_{ex} in *B. saida* suggests energy demanding processes other than locomotion being elevated during maximum performance under high PCO_2 levels with downstream effects for swimming capacity. One potential oxygen demanding source interfering with exercise under high PCO_2 conditions may be the gill. Gill tissue has been found to increase in mass in *G. morhua* during acclimation to 2200 µatm (Kreiss et al. 2015), possibly indicating upregulated acid-base regulation mechanisms due to a

reduced outward directed diffusion gradient. Similar to my results (publication I & II), Kreiss et al. (2015) did not detect an impact of elevated PCO_2 levels on the whole-animal SMR (see paragraph 4.1). Yet, potential cascading effects might be amplified when the organism is operating at maximum capacity resulting in constraints in the swimming performance of *B. saida*. Whole-animal parameters obtained during the present PhD project, however, represent an ensemble of organismic cost and therefore do not allow a precise identification of upregulated mechanisms on lower organismic levels. Accordingly, further studies (e.g. involving blood chemistry measurements in exercising fish) are vitally needed to unravel physiological mechanisms causing elevated aerobic costs under near-future PCO_2 conditions.

Besides a reduction in maximum swimming speeds, hypercapnia caused behavioural impairments in *B. saida*: Absolute lateralization (preference to turn to one side at the end of a maze) of *B. saida* was found to be reduced at high PCO_2 levels (Schmidt et al. 2017). The specialization of fish to flee into one preferred direction is considered as an indicator for predator avoidance efficiency (Dadda et al. 2010). Accordingly, compromised maximum swimming capacities and predator avoiding efficiencies strongly suggest impaired fitness of *B. saida* under near-future ocean acidification conditions especially in light of a lower vulnerability of the invading predator *G. morhua* to high PCO_2 levels (see paragraph 4.2). These constraints, therefore, can be expected to be most relevant for the survival of *B. saida* during episodes of prolonged predator exposure, such as potential spawning migrations.

So far, I evaluated the maximum performance of *B. saida* based on unfed specimens, assuming that aerobic performance of food-deprived specimens following exhaustive swimming tests represents this species' overall maximum aerobic capacities; a common approach in respiration physiology (compare e.g. Norin and Clark 2016). Depending on behavior and foraging strategies, however, aerobic capacities of fish species can be specialized on different purposes (Clark et al. 2013): While highly active, pelagic species such as salmonids reach their highest possible $\dot{M}O_2$ by performing exhaustive exercise, the cardiopulmonary system of benthic ambush predators evolved to support higher MMRs evoked by the digestion (MMR_{dig}) of large meals (Fu et al. 2009). With its moderately active lifestyle (Lønne and Gulliksen 1989), *B. saida* is metabolically situated between

these extremes with unknown potential prioritisations. The MMR_{ex} of unfed *B. saida* following an exhaustive swim test (CAT), however, was higher than the MMR_{dig} of resting fish voluntarily fed to satiation acclimated to both 0 (63.7 %) and 6°C (12.4 %) (publication III). Likewise, when substracting baseline metabolism and comparing the energetic investment for exercise (AS_{ex}) with the investment for digestion (AS_{dig}), the AS_{ex} exceeded the AS_{dig} at both temperatures (69.7 % at 0°C, 60.3 % at 6°C). As postprandial elevated $\dot{M}O_2$ predominantly represents costs involved in food assimilation (compare Chabot et al. 2016b) and thereby the cost for growth, the low AS_{dig} of *B. saida* (e.g. compared to an approximately fourfold higher AS_{dig} of the faster growing *G. morhua*, Chabot et al. 2016b) reflects this species' low growth potential (Laurel et al. 2015, publication I) with a maximum body size of only 40 cm (Ajiad et al. 2011) at age seven (Bradstreet et al. 1986). Accordingly, while rapidly growing species with comparably large maximum body sizes evolved to minimize the time at vulnerable size (Hunt von Herbing and White 2002), *B. saida* relies on hiding strategies (under-ice crevices at age 1 and 2, Lønne and Gulliksen 1989, and cold, deep water layers as adults, Hop and Gjøsæter 2013) in order to avoid predation considering their low growth potential combined with a moderate swimming capacity.

Before prematurely concluding that MMR_{ex} depicts the maximum possible $\dot{M}O_2$ of *B. saida*, however, it has to be considered that exercise and digestion often occur simultaneously in the field (Hicks and Bennett 2004, Thorarensen and Farrell 2006), especially at low Arctic water temperatures causing prolonged SDA durations (Hop et al. 1995). Excess aerobic capacities visible in the capacity to additively combine different metabolic demands (elevated MMR_{ex+dig} compared to MMR_{ex}) enables *D. labrax* and darkbarbel catfish (*Peltebagrus vachelli*) to maintain their maximum swimming speed during parallel ongoing digestive processes (Dupont-Prinet et al. 2009, Jourdan-Pineau et al. 2009, Li et al. 2010). Other species, such as *O. mykiss*, chinook salmon (*Oncorhynchus tshawytscha*) and goldfish (*Carassius auratus*) do not reveal the capacity to increase maximum aerobic performance during periods of coinciding activity and digestion. In these species, limitations of the cardiopulmonary system entail metabolic prioritizations in fed fish performing exercise, visible in a reduced U_{crit} (Alsop and Wood 1997, Thorarensen and Farrell 2006, Pang et al. 2011). The fact that both highly active species and rather poor swimmers express similar metabolic strategies when performing exercise after feeding

implies that metabolic strategies during concomitant high oxygen demanding processes cannot necessarily be derived from species-specific traits such as swimming performance.

The MMR of *B. saida* did not differ between fed and unfed specimens at 0°C, resulting in a slightly reduced U_{gait} and U_{crit} (publication III). Constraints on swimming performance during simultaneously elevated oxygen demands in the gastrointestinal tract imply that the cardiopulmonary system of *B. saida* reached its maximum capacity at this temperature (Alsop and Wood 1997). Further, the elevated blood flow to the digestive system could not arbitrarily be reallocated suggesting an oxygen limitation in red muscle tissue that caused the observed reduction in swimming performance (Thorarensen and Farrell 2006).

At an elevated acclimation temperature (6°C), in contrast, *B. saida* revealed a significantly higher MMR_{ex+dig} (0.1219 µmol O_2 min^{-1} g^{-1}) compared to MMR_{ex} (0.1033 µmol O_2 min^{-1} g^{-1}). While the capacity to elevate MMR is considered a precondition to maintain maximum swimming performance during simultaneous oxygen demands from the digestive system (Jourdan-Pineau et al. 2009, Li et al. 2010), fed *B. saida* even revealed a significantly higher U_{gait} (unfed: 3.4 BL sec^{-1}, fed: 3.8 BL sec^{-1}) and U_{crit} (unfed: 3.7 BL sec^{-1}, fed: 4.2 BL sec^{-1}). Accordingly, digestion did not result in an impairment of the E_{max} of *B. saida*. The improved swimming performance detected in fed individuals may be related to excess dietary nutrients available for immediate energy production (Alsop and Wood 1997). In turn, unfed specimens were not able to fully exploit their muscular capacities at 6°C. Accordingly, swimming performance was likely limited by energy shortage of the skeletal muscle during food deprivation. An oxygen limitation of the swimming muscle as suspected in unfed *B. saida* at 0°C seems unlikely based on the capacity of fed specimens to enhance their MMR_{ex+dig} at 6°C.

The different metabolic strategies of cold- and warm-acclimated *B. saida* show that food-deprived specimens suffer from a higher thermal sensitivity. Accordingly, food deprivation – in addition to high PCO_2 levels – causes an impairment of the swimming performance of *B. saida* at elevated temperatures. Resulting disadvantages for *B. saida* therefore are expected to be amplified under climate-change induced future water conditions predicted to entail reduced prey availabilities (Fossheim et al. 2015). *B. saida*, consequently, will be forced to invest increasing effort into foraging thereby being at higher risk for predation due to both a prolonged exposure period and an impaired swimming performance. Additionally, increasing energetic investment for activity may come at the expense of other

aerobic processes, such as growth and reproduction, thus entailing further fitness constraints.

4.3.2 Anaerobic swimming performance and its ecological relevance

Anaerobic swimming performance is optically manifested as transient burst-and-coast swimming behavior at velocities exceeding U_{gait} (Hemmings 1973). The term burst-and-coast swimming (hereafter referred to as burst swimming) describes an intial sudden acceleration powered by anaerobically fueled white muscle fibres followed by a short phase of motionless gliding (Videler and Weihs 1982). Despite inter- and intraspecific differences, periods of repeated burst-and-coast swimming can only be maintained for several seconds (Beamish 1978) due to a high energetic investment involved (*in vitro* costs at maximum capacity: 126 µmol ATP min^{-1} g^{-1} in *O. mykiss*, compared to maximum costs of red muscle: 42 µmol ATP min^{-1} g^{-1} in skipjack tuna, *Katsuwonus pelamis*) (Moyes and West 1995) resulting in a rapid exploitation of white muscle reserves. Within the first seconds, burst-type exercise is fueled with ATP and phosphocreatine (Dobson et al. 1987) stored in the white muscle. Immediately afterwards, ATP is generated from glycogen, resulting in an accumulation of lactate and metabolic protons (among other end-products) (Dobson et al. 1987). Accordingly, subsequent episodes of burst-type swimming depend on efficient replenishments of white muscle energy stores (compare review by Milligan 1996) and may be increasingly limited by an accumulation of metabolic end-products (compare review by Kieffer 2000).

In the present study, anaerobic swimming capacities of *B. saida* were estimated from the number of burst events recorded in two different swimming protocols. Swimming during U_{crit} protocols is predominantly powered by aerobic fuels, while the rapid velocity increase during CAT protocols elicites a larger recruitment of white muscle fibres. Accordingly, the maximum number of bursts (BC_{max}) of *B. saida* obtained by U_{crit} tests (19 bursts at 0°C, 9 bursts at 6°C extrapolated to a measurement period of 50 sec) (publication II) was lower than during the CAT (24 bursts at 0°C, 25 bursts at 6°C) (publication III) (21 and 64 % lower at 0 and 6°C, respectively). In order to evaluate glycolytic performance of *B. saida*, the following discussion focusses on the results obtained from the CAT in publication III.

The BC_{max} of the Arctic endemic *B. saida* revealed by a CAT protocol was severalfold lower (25 bursts during 50 sec at 6°C) (publication III) compared to the temperate *D. labrax*

(~ 140 bursts extrapolated to a measurement period of 50 sec at 23°C) (Marras et al. 2010). A few specimens even did not reveal burst swimming at all (n = 4 out of 42 different swimming trials with most individuals measured in three swimming rounds, publication III). The low capacity for burst-type exercise may be attributed to a low potential for anaerobic glycolysis as detected in Antarctic fish that also express limited burst performance (Dunn and Johnston 1986, Davison et al. 1988). Based on slightly higher resting blood lactate levels, however, Whiteley et al. (2006) ascribed the Arctic *B. saida* a higher glycolytic performance compared to Antarctic species. Pörtner (2002b) suggested a connection between low glycolytic capacities and cold-adaptation in polar species. The higher glycolytic performance of Arctic compared to Antarctic fish species, therefore, might be attributed to the substantially shorter period of stable low habitat temperatures in the Arctic (0.7 – 2 million years since the ice-cover formation in the Arctic, Eastman 1997) compared to the Antarctic (oceanographic isolation of Antarctica occurred 22 – 25 million years ago, Eastman 1997) (Whiteley et al. 2006).

Kieffer et al. (1994) found *O. mykiss* acclimated to elevated temperatures to use a higher fraction of muscle glycogen stores during burst exercise, entailing a higher concentration of metabolic end-products in the blood. Nevertheless, the clearance of lactate and metabolic protons occurred within the same time frame as recorded under control temperatures, resulting in comparable anaerobic swimming capacities of cold and warm-acclimated fish (Kieffer et al. 1994). Likewise, acclimation temperature did not affect the burst swimming performance of *B. saida* (publication II & III). A depression of burst performance in response to high PCO_2 exposure, however, was detected (publication II), in line with the overall reduced swimming capacity of *B. saida* in hypercapnic treatments (see paragraph 4.3.1). In a similar manner, Kreiss et al. (in prep.) suggest an impairment of anaerobic performance of *G. morhua* acclimated to 2200 µatm CO_2 reflected in reduced lactate dehydrogenase capacities. In addition to elevated PCO_2 levels, food deprivation also resulted in a reduced burst performance (publication III) likely attributed to reduced glycogen stores as detected in food-deprived *O. mykiss* (Scarabello et al. 1991, Kieffer and Tufts 1998). Moreover, the sensitivity of glycogen stores to food shortage is higher in juveniles (as considered in the present study) than in adults (Kieffer and Tufts 1998), likely amplifying the impairment of anaerobic swimming capacities.

Burst swimming performance plays an important role for predator avoidance and in some species for the achievement of prey capture (Domenici and Blake 1997). Considering

limited escape capacities of species preyed upon by *B. saida* (such as amphipods and copepods, Lønne and Gulliksen 1989), burst-type exercise of *B. saida* is expected to be predominantly involved during predator encounters. The limited burst performance detected in *B. saida*, further, is in line with this species' strategy to outlast predator encounters at hardly accessible places or in areas not hospitable for many predator species as discussed above (see paragraph 4.3.1). Combined with the overall reduced maximum performance of *B. saida* under future conditions, impairment of burst performance at predicted PCO_2 levels and under conditions of food deprivation likely increases the vulnerablitiy of *B. saida* to predation in the future.

5 Conclusions

Conclusions drawn from long-term exposure of Polar cod (*Boreogadus saida*) and Atlantic cod (*Gadus morhua*) to future ocean water conditions are presented based on the objectives stated in paragraph 1.5:

OBJECTIVE 1: DO ROUTINE WHOLE-ANIMAL PARAMETERS OF JUVENILE *B. SAIDA* AND *G. MORHUA* CHRONICALLY EXPOSED TO OAW SCENARIOS INDICATE LIMITATIONS OF SPECIES-SPECIFIC ACCLIMATION CAPACITIES?

Routine parameters of *B. saida* and *G. morhua* recorded in the present study comprised the standard metabolic rate (SMR), growth performance and several body index parameters (CF, HSI, GSI). The Arctic endemic *B. saida* showed multiple signs of cold-adaptation (high food conversion efficiency (FCE), low costs for digestion (SDA magnitude) as a result of low food intake (F), low SMR and high gonadosomatic index (GSI) at 0°C). At the highest investigated acclimation temperature (8°C), in turn, *B. saida* revealed limited compensation capacities perceivable in an elevated SMR that result in an impaired growth performance. Accordingly, I suggest 8°C to represent the upper pejus temperature (T_{pej}) of juvenile *B. saida* originating from Svalbard waters, while more subtle organismic limitations (decreasing condition factor (CF) and non-increasing F) already appeared at acclimation temperatures > 3°C. In contrast, all whole-animal routine parameters of the invading boreal *G. morhua* indicate an increasing performance with rising temperature within the investigated thermal range (3 – 16°C), while cold-induced limitations were detected at 3°C, visible in a reduced FCE. Accordingly, 3°C was classified as the lower T_{pej} of juvenile *G. morhua*. At comparable acclimation temperatures (3 and 8°C), the SMR of *B. saida* was higher than the one of *G. morhua*. While higher SMR values at 3°C likely represent costs associated with cold-adaptation (such as higher mitochondrial densities or enhanced rates of mitochondrial substrate oxidation), the SMR of *B. saida* at 8°C is further elevated due to an enhanced mitochondrial proton leak.

The high degree of adaptation to 0°C compared to higher temperatures reflects current thermal habitat conditions of *B. saida* that are distributed in under-ice habitats (Lønne and Gulliksen 1989) as well as in deep water layers of fjords both characterized by subzero temperatures (David et al. 2015, Madsen et al. 2015). Ongoing climate change causes a decrease in the sea ice cover. Accordingly, *B. saida* will not only be progressively deprived of low-temperature niches and shelters in surface waters, moreover, with melting sea ice

suitable spawning grounds are expected to become sparse likely entailing tremendous impacts on this species' abundance. At the same time, the decrease in sea ice and concomitantly rising water temperatures opens new habitats for juvenile *G. morhua*. Congruent with my findings of an increasing whole-animal performance of juvenile *G. morhua* above 3°C, *G. morhua* was found in ice-free surface layers of 4.0 – 6.0°C. Moreover, even small temperature increases in deep water may be sufficient to prolong predatory excursions of *G. morhua*, further impairing the abundance of *B. saida*. Besides *G. morhua*, additional boreal fish species that may pose both predation pressure (e.g. haddock, *Melanogrammus aeglefinus*, Renaud et al. 2012) and competition for *B. saida* (e.g. capelin, *Mallotus villosus*, Hop and Gjøsæter 2013) are expanding their distribution northwards into Svalbard waters. Due to the progressive loss in sea ice cover and associated declining potential spawning grounds, as well as a distinctly increasing performance of *G. morhua* and further boreal fish species with rising water temperatures, thermal habitat conditions approaching 8°C may have detrimental effects on *B. saida*. Consequently, a northeastward shift in the distribution of *B. saida* is already observed (Eriksen et al. 2015). Compared to thermal effects, the impact of elevated PCO_2 levels on routine parameters of both species was rather subtle. In contrast to the growth performance of *B. saida*, growth of *G. morhua* was not affected by hypercapnic conditions, indicating a lower vulnerablitiy of the latter species to future ocean acidification scenarios potentially because *G. morhua* is facing a higher variability in abiotic conditions in its natural habitat (Neuenfeldt et al. 2009). Nevertheless, a significantly elevated hepatosomatic index (HSI) of *G. morhua* under hypercapnia at 16°C may indicate a higher thermal sensitivity of this species under high compared to control PCO_2 conditions. Differences in species-specific sensitivities to future ocean acidification and warming scenarios indicate a higher competitive strength of the boreal invading *G. morhua* compared to the native *B. saida*.

OBJECTIVE 2: WILL LONG-TERM OAW EXPOSURE AFFECT MAXIMUM PERFORMANCE CAPACITIES OF JUVENILE *B. SAIDA*?

Maximum aerobic capacities of *B. saida* evoked by exercise (MMR_{ex}) increased with acclimation temperature with signs of a moderate compensation capacity. Combined with the thermal pattern of SMR, the MMR_{ex} describes a peak aerobic scope (AS_{ex}) of *B. saida* at 6°C. Peak AS_{ex}, however, did not translate into an elevated swimming performance.

Instead, maximum sustainable swimming speed (U_{gait}) (exclusively fueled by aerobic metabolism) of *B. saida* revealed a weak increase throughout the range of acclimation temperatures, while the critical swimming speed (U_{crit}) (partially fueled by anaerobic metabolism) was unaffected by different thermal conditions. Accordingly, swimming performance is maintained throughout a surprisingly broad thermal range, potentially in order to support spawning migrations across areas with diverging temperatures. Nevertheless, the decrease in maximum swimming efficiency (E_{max}) ≥ 6°C under normocapnic conditions and ≥ 3°C under hypercapnic conditions indicates performance limitations with increasing habitat temperature, as well as a higher thermal sensitivity under concomitantly elevated PCO_2 levels. Near-future PCO_2 conditions further caused a distinct depression in both U_{gait} and U_{crit}, despite a significant increase in MMR_{ex}. The resulting reduction in E_{max} indicates vulnerability of maximum performance of *B. saida* to future ocean acidification scenarios. Accordingly, high PCO_2 levels entail impaired sustainable migration capacities as well as a reduced capacity for predator avoidance of *B. saida* potentially resulting in reduced survival rates, especially when considering a lower vulnerability of *G. morhua* to near-future PCO_2 conditions as detected in resting parameters.

OBJECTIVE 3: DOES FOOD DEPRIVATION AFFECT MAXIMUM PERFORMANCE OF *B. SAIDA* AT DIFFERENT ACCLIMATION TEMPERATURES?

B. saida is a moderately active (Lønne and Gulliksen 1989) and comparably slow growing species (Hop and Gjøsæter 2013, Laurel et al. 2015). Therefore, *B. saida* commonly avoids predators by hiding in hardly accessible habitats or locations characterized by low temperatures that most of its predators cannot tolerate for extended periods (Lønne and Gulliksen 1989, Hop and Gjøsæter 2013). Nevertheless, little is known about potential metabolic prioritizations of *B. saida*. The maximum metabolic rate following maximum swimming performance in unfed *B. saida* (MMR_{ex}) exceeded the maximum $\dot{M}O_2$ evoked by digestion (MMR_{dig}) in resting specimens, reflecting its low energetic investment for growth. Considering low habitat temperatures of *B. saida*, energetic demands for exercise and digestion are expected to occur simultaneously. Maximum respiratory capacities, however, were not enhanced when fish were exercised following feeding (MMR_{ex+dig}) compared to exercised food-deprived specimens at 0°C suggesting maximum

cardiopulmonary capacities of *B. saida* to be reached at this temperature. The resulting depression in swimming performance (both U_{gait} and U_{crit}) in fed specimens indicated that an elevated blood flow to the digestive system caused oxygen limitation in the red swimming muscle. At 6°C, in contrast, the MMR_{ex+dig} of *B. saida* significantly exceeded the MMR_{ex}. While one could assume that the rise in MMR_{ex+dig} reflects additive energetic demands of swimming performance and digestion, the swimming capacity of freshly fed fish was even significantly higher compared to unfed individuals, indicating a nutrient limitation in red swimming muscle after extended periods of food deprivation. Hence, different metabolic strategies of cold- and warm-acclimated *B. saida* suggest that food deprivation in combination with elevated water temperatures causes a distinct fitness impairment.

Accordingly, detrimental effect on maximum swimming performance of *B. saida* were caused by food deprivation at elevated water temperatures in addition to elevated PCO_2 conditions. Both a reduction in prey abundance for *B. saida* and increasing PCO_2 levels are projected to occur concomitantly with further rising water temperatures, thereby likely amplifying detrimental effects on this species' swimming performance. Reduced future prey availabilities, however, will most likely force *B. saida* to invest more effort into foraging going along with an enhanced risk for predation due to prolonged periods of predator exposure combined with an impaired U_{crit}. Further, higher investment into foraging activity may entail trade-offs for other aerobic processes with potential detrimental effects on abundance.

OBJECTIVE 4: HOW DOES BURST-AND-COAST SWIMMING PERFORMANCE OF JUVENILE *B. SAIDA* RESPOND TO FUTURE OCEAN WATER CONDITIONS? WHAT IS THE ROLE OF BURST-AND-COAST SWIMMING MODE FOR THIS SPECIES IN AN ECOLOGICAL CONTEXT?

In the present study, the number of burst-and-coast swimming events was considered as an estimate for anaerobic swimming capacities of *B. saida*. Compared to more active species, *B. saida* revealed a low burst performance and thereby a low estimated contribution of anaerobic metabolism during maximum swimming performance. Similar observations are documented for Antarctic fish species that revealed low capacities for anaerobic glycolysis (Dunn and Johnston 1986, Davison et al. 1988). Elevated acclimation temperatures did not

affect the burst swimming performance of *B. saida*. Near-future high PCO_2 conditions and food deprivation, however, resulted in a reduction of the burst capacity in this species.
In general, fish apply the burst-and-coast swimming mode for efficient prey capture as well as an escape reaction during predator encounters. As prey species of *B. saida* are rather slowly moving organisms, burst swimming performance is expected to be of highest importance during predator encounters in order to increase survival chances. The low burst capacity of *B. saida*, therefore, is in line with its hiding strategy as described above. Nevertheless, a reduction in burst swimming in addition to an overall reduced swimming performance under near-future water conditions, represents distinct fitness impairments of *B. saida*. A progressive loss of shelter with decreasing sea ice, therefore, may have severe impacts on survival rates and thereby the abundance of *B. saida*.

Overall, while neglecting potential trans-generational adaptation capacities, the results of this thesis suggest a decreased fitness of juvenile *B. saida* from Svalbard waters under projected ocean water conditions: The SMR of *B. saida* indicated limitations in the capacity to acclimate to water temperatures as high as 8°C, along with a reduced growth performance. Hypercapnic conditions further impaired growth. The maximum sustainable (U_{gait}) as well as the critical swimming performance (U_{crit}) and its energetic efficiency (E_{max}) were reduced under near-future PCO_2 conditions, while food deprivation proved detrimental for U_{gait} and U_{crit} of *B. saida* only at elevated temperatures. Further, burst swimming that is assumed to represent an important predator avoidance mechanism of *B. saida* was as well impaired by both ocean acidification and food deprivation scenarios. Especially in light of an improving performance of invading boreal predator species such as *G. morhua* with rising water temperatures and their lower vulnerability to ocean acidification, the competitive strength of the endemic *B. saida* in Svalbard waters is strongly expected to decrease.

6 Synthesis

The present thesis contributed to unravel physiological responses of Polar cod (*Boreogadus saida*) to projected changes in abiotic and biotic conditions, thereby aiming to identify limitations in the acclimation capacity of this species to future ocean water conditions. The ongoing climate change, however, also goes along with changes in physical oceanographic processes of Arctic fjords with potential additional impacts on the fate of *B. saida*. In the present chapter, two independent scenarios for future shifts in oceanographic patterns with potential implementations for *B. saida* are briefly presented that go beyond the specific objectives this thesis was focusing on.

Hydrodynamics and their future changes in response to ongoing climate change are differing vastly between the fjords along the west coast of Svalbard depending on geographical location (Promińska et al. 2017), connection to coastal waters and topography (Cottier et al. 2010), in addition to more variable physical processes such as wind forcing and freshwater discharge (Promińska et al. 2017).

Fjords in the southwest of Svalbard (e.g. Hornsund) are seasonally isolated from Atlantic water masses transported by the West Spitsbergen Current (WSC) due to the cold Sørkapp Current that passes the southern tip of Svalbard and weakens along the West Spitsbergen Shelf (see paragraph 1.2.2) (Promińska et al. 2017). In the northwest of Svalbard, however, fjords characterized by broad mouths and without entrance sill are experiencing a distinct exchange with Atlantic water from the WSC (Promińska et al. 2017). The intrusion of Atlantic water masses is amplified by seasonal wind forcing that facilitates cross-shelf exchange (Cottier et al. 2007). Accordingly, a progessive atlantification of water masses throughout all layers was observed in Kongsfjorden in summers between 2001 and 2015 (FIG. 20) (Promińska et al. 2017). The continuous warming of the WSC further results in a steady increase in mean temperature in the main part of the fjord (1.80 and 3.96°C in 2001 and 2015, respectively) (Promińska et al. 2017). *B. saida* inhabits the deep water layers of Kongsfjorden yearround (e.g. Nahrgang et al. 2010). An enhanced heat delivery even in the deep water in recent years may act as a warming cue causing the cold-adapted *B. saida* – at least seasonally – to leave Kongsfjorden. Future warming of the WSC combined with a reduced ice production that entails similar densities of both fjord and Atlantic water, thereby facilitating the entrance of Atlantic water masses (Nilsen et al. 2008) as well as projected more extreme wind forcing (Delworth and Dixon 2000), may cause water

temperatures of Kongsfjorden to permanently exceed thermal conditions tolerated by *B. saida*. These conditions further support the settlement of boreal species as projected by Drinkwater (2005).

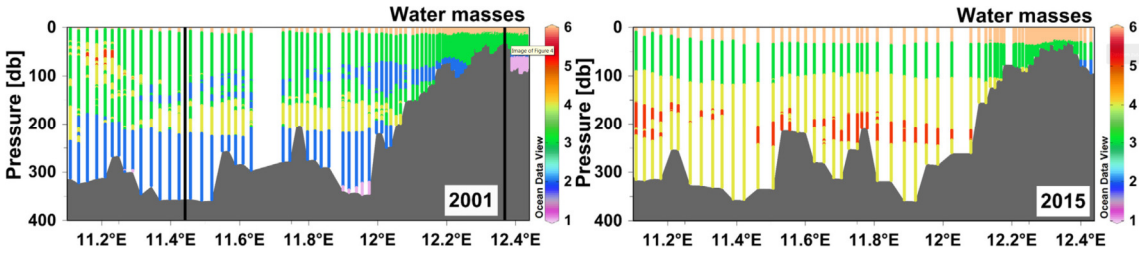

FIGURE 20. Comparison of water masses along fjord axis in Kongsfjorden in 2001 (left) and 2015 (right). Orange = surface water, green = intermediate water, yellow = transformed Atlantic water, blue = local water, pink = winter cooled water, red = Atlantic water (Promińska et al. 2017).

In contrast, fjords associated with larger fjord systems and thereby located further inland, such as Billefjorden (inner northeastern branch of the Isfjorden system) are less exposed to Atlantic water masses. In addition, the water body of Billefjorden is separated from the outer water body by two high entrance sills (outer and inner sill at depth 70 and 50 m, respectively) (Nilsen et al. 2008). In summer, sill fjords are commonly stratified in three layers due to thermohaline processes (Farmer and Freeland 1983): a surface layer characterized by freshwater, an intermediate layer and a high saline cold deep water layer below the sill depth (Cottier et al. 2010). Cooling of surface water and ice production with the associated brine release causes a suspension of the thermohaline stratification in winter (Haarpaintner et al. 2001), resulting in a re-oxygenation of bottom water layers (Cottier et al. 2010). Rising atmospheric temperatures are inducing an increasing freshwater runoff from melting tidewater glaciers, potentially intensifying the thermohaline stratification and thereby preventing the seasonal exchange and the associated replenishment of oxygen in the deep water layer of Billefjorden. In October 2018, the oxygen content of the deep water layer was 20 % below atmospheric oxygen saturation (Mark in prep.) due to isolation of the bottom water body (FIG. 21). *B. saida* is known to inhabit cold deep water layers of sill fjords (Madsen et al. 2015) and is frequently found in Billefjorden in autumn (Mark 2015, Mark in prep.). A prolonged or even interannual absence of deep water exchange would likely entail a progressive decrease in PO_2 thereby only supporting survival of *B. saida* for limited periods, depending on their PO_{2crit}.

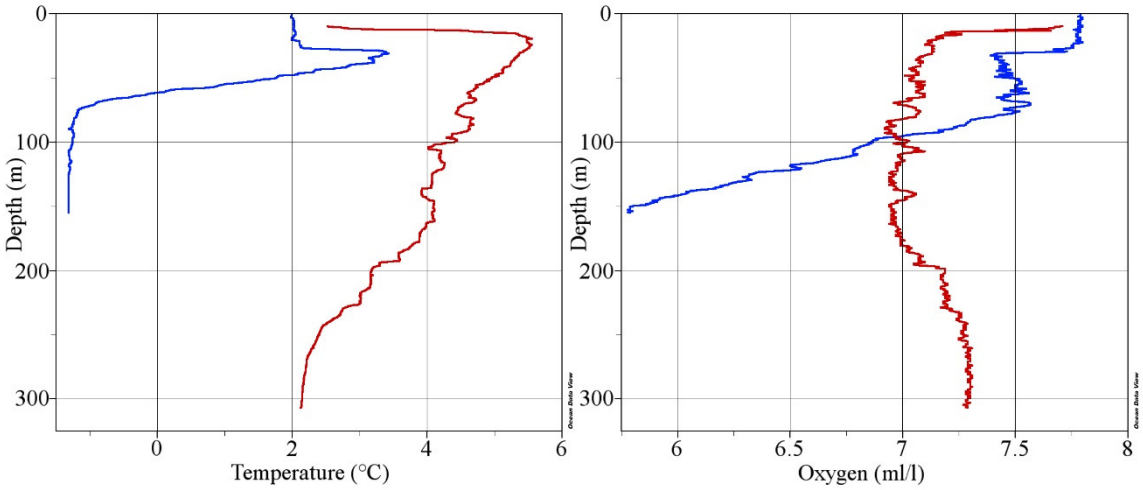

FIGURE 21. Comparison of temperature (left) and PO_2 profiles (right) from Kongsfjorden (red) and Billefjorden (blue) in October 2018 (Mark in prep.).

Accordingly, while results of the present study indicate that direct effects of climate change likely compromise the physiological performance of *B. saida* under future water conditions, local effects may additionally cause a progressive decline in suitable habitats for this species in the vicinity of Svalbard.

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Appendix

TABLE A1a: Data of Polar cod *Boreogadus saida* of publication I (means ± SEM).

TABLE A1b: Data of Atlantic cod *Gadus morhua* of publication I (means ± SEM).

TABLE A2: Data of publication II (means ± SEM).

TABLE A3: Data of publication III (means ± SEM).

A4: Publication IV (Schmidt et al. 2017).

A5: Publication V (Leo et al. 2017).

TABLE A1a: Data of Polar cod *Boreogadus saida* of publication I (means ± SEM). SGR = specific growth rate, F = daily food consumption, FCE = food conversion efficiency, CF = condition factor, HSI = hepatosomatic index, GSI = gonadosomatic index, SMR = standard metabolic rate, SF = stomach filling. N = 12, unless stated otherwise by numbers in brackets. For data on individuals, see https://doi.pangaea.de/10.1594/PANGAEA.867390).

Species	Temp. (°C)	pCO_2 (µatm)	Incubation time (days)	Mort. (%)	SGR (% d^{-1})	F (g d^{-1} BW^{-1})	FCE	CF	HSI (%)	GSI male (%)	GSI female (%)	SMR (µmol O_2 min^{-1} g^{-1})	SF (% stomach wt.)
Boreogadus saida	0	390	111 – 116	0.00	0.3861 ± 0.0272	0.0037 ± 0.0002	1.23 ± 0.08	0.73 ± 0.03	5.49 (11) ± 0.37	6.80 (9) ± 1.84	1.68 (2) ± 1.33	0.0542 (5) ± 0.0028	52.51 (11) ± 4.60
	0	1170	111 – 124	0.00	0.3210 ± 0.0379	0.0033 ± 0.0002	1.13 ± 0.14	0.68 ± 0.03	4.90 ± 0.43	7.77 (10) ± 2.03	2.91 (1)	0.0504 (5) ± 0.0050	53.03 ± 4.75
	3	390	102 – 116	0.00	0.4491 (11) ± 0.0512	0.0049 (11) ± 0.0004	1.00 (11) ± 0.08	0.70 (11) ± 0.03	5.52 (10) ± 0.31	4.59 (10) ± 1.20	- (0)	0.0500 (5) ± 0.0016	49.09 (10) ± 6.44
	3	1170	110 – 119	0.00	0.3742 ± 0.0442	0.0045 ± 0.0003	0.93 ± 0.06	0.69 ± 0.01	6.15 ± 0.43	5.51 (8) ± 1.19	4.50 (3) ± 0.68	0.0530 (6) ± 0.0061	49.76 (6) ± 7.63
	6	390	113 – 118	0.00	0.5093 ± 0.0533	0.0050 ± 0.0003	1.05 ± 0.08	0.65 ± 0.03	6.05 ± 0.29	3.59 (10) ± 0.60	2.39 (2) ± 0.60	0.0574 (5) ± 0.0030	35.34 (12) ± 6.61
	6	1170	113 – 130	0.00	0.4197 ± 0.0621	0.0045 ± 0.0003	0.94 ± 0.08	0.64 ± 0.01	5.78 (10) ± 0.50	3.80 (7) ± 0.76	2.52 (3) ± 0.12	0.0546 (6) ± 0.0028	46.67 (10) ± 7.05
	8	390	96 – 117	8.33	0.3486 ± 0.0424	0.0048 ± 0.0004	0.71 ± 0.06	0.60 ± 0.02	6.20 (11) ± 0.34	1.41 (10) ± 0.18	1.59 (1)	0.0828 (6) ± 0.0067	31.02 (11) ± 5.47
	8	1170	58 – 125	18.18	0.3447 (11) ± 0.0573	0.0050 (11) ± 0.0002	0.70 (11) ± 0.10	0.63 (11) ± 0.02	5.99 (8) ± 0.62	1.66 (7) ± 0.37	1.78 (1)	0.0757 (4) ± 0.0060	34.93 (8) ± 5.39

TABLE A1b: Data of Atlantic cod *Gadus morhua* of publication I (means ± SEM). SGR = specific growth rate, F = daily food consumption, FCE = food conversion efficiency, CF = condition factor, HSI = hepatosomatic index, GSI = gonadosomatic index, SMR = standard metabolic rate, SF = stomach filling. N = 12, unless stated otherwise by numbers in brackets. For data on individuals, see https://doi.pangaea.de/10.1594/PANGAEA.867390).

Species	Temp. (°C)	PCO_2 (µatm)	Incubation time (days)	Mort. (%)	SGR (% d^{-1})	F (g d^{-1} BW^{-1})	FCE	CF	HSI (%)	GSI male (%)	GSI female (%)	SMR (µmol O_2 min^{-1} g^{-1})	SF (% stomach wt.)
Gadus morhua	3	390	121 – 133	0.00	0.2935 ± 0.0568	0.0050 ± 0.0004	0.65 ± 0.12	0.73 ± 0.02	4.36 ± 0.40	0.08 (5) ± 0.00	0.35 (5) ± 0.02	0.0317 ± 0.0019	16.51 (6) ± 5.61
	3	1170	19 – 120	8.33	0.3070 (11) ± 0.0312	0.0052 (11) ± 0.0005	0.66 (11) ± 0.05	0.74 (11) ± 0.02	3.34 (11) ± 0.39	0.10 (3) ± 0.02	0.32 (6) ± 0.02	0.0274 (11) ± 0.0023	7.67 (7) ± 3.84
	8	390	77 – 128	8.33	0.6817 ± 0.0535	0.0084 ± 0.0007	1.01 ± 0.05	0.78 ± 0.03	4.82 (7) ± 0.70	- (0)	0.36 (6) ± 0.02	0.0447 (10) ± 0.0031	0.33 (5) ± 0.20
	8	1170	15 – 120	25.00	0.6146 (9) ± 0.0562	0.0086 (9) ± 0.0007	0.88 (9) ± 0.05	0.77 (9) ± 0.02	4.94 (9) ± 0.25	0.07 (3) ± 0.01	0.37 (6) ± 0.02	0.0423 (9) ± 0.0025	0.82 (9) ± 0.28
	12	390	2 – 132	33.33	0.7818 (9) ± 0.0566	0.0104 (9) ± 0.0007	0.95 (9) ± 0.06	0.81 (9) ± 0.03	4.65 (6) ± 0.61	0.14 (1)	0.36 (4) ± 0.03	0.0472 (7) ± 0.0020	1.41 (5) ± 0.72
	12	1170	31 – 124	8.33	0.8485 (11) ± 0.0445	0.0106 (11) ± 0.0010	0.99 (11) ± 0.04	0.76 (11) ± 0.02	5.85 (11) ± 0.49	0.13 (4) ± 0.05	0.27 (7) ± 0.08	0.0524 (11) ± 0.0027	2.68 (10) ± 1.02
	16	390	4 – 132	16.67	0.8908 (10) ± 0.0777	0.0120 (10) ± 0.0008	0.97 (10) ± 0.06	0.84 (10) ± 0.02	5.69 (5) ± 0.21	0.11 (3) ± 0.04	0.27 (2) ± 0.21	0.0716 (5) ± 0.0027	3.05 (5) ± 0.87
	16	1170	95 – 132	0.00	0.9504 (11) ± 0.0604	0.0104 (11) ± 0.0007	1.00 (11) ± 0.03	0.84 (11) ± 0.03	8.32 (5) ± 0.26	0.13 (1)	0.42 (4) ± 0.05	0.0640 (5) ± 0.0018	1.44 (5) ± 1.04

TABLE A2: Data of publication II (means ± SEM). SMR = standard metabolic rate, MMR = maximum metabolic rate, AS = aerobic scope, U_{gait} = gait transition speed, U_{crit} = critical swimming speed, E_{max} = swimming efficiency, BC_{max} = maximum burst count, BC_{tot} = total number of bursts, TSB = time between U_{gait} and U_{crit} ("time spent bursting"), $TSB_{anaerob}$ = proportion of anaerobic metabolism during the period between U_{gait} and U_{crit}. Numbers in brackets represent n-values. For data on individuals, see https://doi.pangaea.de/10.1594/PANGAEA.889447.

Species	Temp. (°C)	PCO_2 (µatm)	Incubation time (days)	SMR (µmol O_2 min^{-1} g^{-1})	MMR (µmol O_2 min^{-1} g^{-1})	AS (µmol O_2 min^{-1} g^{-1})	U_{gait} (BL sec^{-1})	U_{crit} (BL sec^{-1})	E_{max} (BL g $µmol^{-1}$)	BC_{max}	BC_{tot}	TSB (sec)	$TSB_{anaerob}$ (%)
Boreogadus saida	0	390	116 – 117	0.0537 (5) ± 0.0030	0.0970 (5) ± 0.0082	0.0432 (5) ± 0.0088	3.04 (5) ± 0.19	3.49 (5) ± 0.19	1968.1 (4) ± 51.9	11.4 (5) ± 3.4	24.0 (5) ± 6.1	2784 (5) ± 679	1.7 (5) ± 1.0
	0	1170	124	0.0505 (5) ± 0.0042	0.0870 (5) ± 0.0106	0.0364 (5) ± 0.0126	2.94 (5) ± 0.09	3.32 (5) ± 0.14	2444.0 (5) ± 352.0	7.6 (5) ± 1.6	18.1 (5) ± 3.4	2280 (5) ± 251	0.8 (5) ± 0.1
	3	390	102 – 116	0.0503 (4) ± 0.0042	0.0995 (4) ± 0.0198	0.0305 (3) ± 0.0020	3.27 (6) ± 0.12	3.51 (6) ± 0.16	2187.4 (4) ± 333.3	19.5 (6) ± 3.4	27.7 (6) ± 5.6	1430 (6) ± 348	2.3 (6) ± 0.4
	3	1170	118 – 119	0.0525 (6) ± 0.0060	0.1319 (6) ± 0.0157	0.0778 (5) ± 0.0172	2.95 (5) ± 0.02	3.06 (6) ± 0.15	1460.0 (6) ± 121.1	11.8 (6) ± 2.9	22.7 (6) ± 6.6	1610 (6) ± 343	1.4 (6) ± 0.4
	6	390	118 – 123	0.0592 (5) ± 0.0030	0.1249 (5) ± 0.0033	0.0649 (4) ± 0.0061	3.30 (4) ± 0.24	3.37 (4) ± 0.23	1600.5 (4) ± 85.2	5.2 (5) ± 1.6	8.7 (5) ± 4.0	768 (5) ± 436	1.2 (5) ± 0.6
	6	1170	129 – 130	0.0589 (6) ± 0.0029	0.1545 (4) ± 0.0049	0.0945 (4) ± 0.0061	3.11 (4) ± 0.15	3.29 (4) ± 0.21	1312.0 (3) ± 71.1	6.2 (5) ± 1.5	12.5 (5) ± 4.7	1104 (5) ± 482	1.1 (5) ± 0.3
	8	390	112 – 117	0.0838 (5) ± 0.0074	0.1333 (5) ± 0.0102	0.0495 (5) ± 0.0083	3.63 (4) ± 0.19	3.88 (4) ± 0.12	1799.7 (4) ± 235.3	11.9 (6) ± 3.6	22.2 (6) ± 9.0	1210 (6) ± 523	1.3 (6) ± 0.4
	8	1170	125	0.0708 (4) ± 0.0059	0.1618 (4) ± 0.0153	0.0911 (4) ± 0.0196	3.28 (4) ± 0.10	3.42 (4) ± 0.15	1300.4 (4) 121.9	4.0 (4) ± 1.2	6.5 (4) ± 1.9	945 (4) ± 280	0.9 (4) ± 0.3

TABLE A3: Data of publication III (means ± SEM). Ration = daily amount of consumed food, SMR = standard metabolic rate, MMR = maximum metabolic rate, AS = aerobic scope, U_{gait} = gait transition speed, U_{crit} = critical swimming speed, E_{max} = swimming efficiency, BC_{max} = maximum burst count, SDA duration = time span of postprandial elevated $\dot{M}O_2$, SDA magnitude = area underneath the curve that is described by the postprandial increase in $\dot{M}O_2$. Numbers in brackets represent n-values. For data on individuals, see https://doi.pangaea.de/10.1594/PANGAEA.889161.

Species	Temp. (°C)	Approach	Incubation time (days)	Ration (% BW^{-1} d^{-1})	SMR (µmol O_2 min^{-1} g^{-1})	MMR (µmol O_2 min^{-1} g^{-1})	AS (µmol O_2 min^{-1} g^{-1})	U_{gait} (BL sec^{-1})	U_{crit} (BL sec^{-1})	E_{max} (BL g μmol^{-1})	BCmax	SDA duration (h)	SDA magn. (µmol O_2 g^{-1})
Boreogadus saida	0	digestion	107	3.4	0.0306 (5) ± 0.0052	0.0545 (4) ± 0.0058	0.0290 (4) ± 0.0065	-	-	-	-	166.5 (4) ± 30.2	146.16 (4) ± 50.59
	0	exercise	71 – 74	-	0.0393 (7) ± 0.0024	0.0892 (4) ± 0.0049	0.0492 (4) ± 0.0060	3.3 (3) ± 0.1	3.6 (3) ± 0.1**	2334.8 (4) ± 214.3	15.8 (4) ± 6.6	-	-
	0	digestion & exercise	88 – 91	2.6	-	0.0853 (6) ± 0.0033	-	3.2 (6) ± 0.1	3.4 (6) ± 0.1	2431.4 (6) ± 126.3	23.7 (6) ± 5.3	-	-
	0/3*	digestion & exercise	103 – 105	2.6	-	0.1145 (6) ± 0.0069	-	3.6 (6) ± 0.1	3.8 (6) ± 0.1	2023.0 (6) ± 161.2	34.0 (6) ± 6.1	-	-
	6/3*	digestion & exercise	98 – 102	4.3	-	0.0994 (8) ± 0.0055	-	3.5 (8) ± 0.1	3.7 (8) ± 0.1	2309.5 (8) ± 173.6	25.6 (8) ± 3.3	-	-
	6	digestion	13	4.4	0.0484 (5) ± 0.0104	0.1012 (3) ± 0.0123	0.0426 (3) ± 0.0024	-	-	-	-	135.3 (3) ± 43.9	169.14 (3) ± 56.27
	6	exercise	81 – 84	-	0.0455 (8) ± 0.0045	0.1137 (8) ± 0.0072	0.0683 (8) ± 0.0064	3.4 (8) ± 0.1	3.7 (8) ± 0.1	2008.0 (8) ± 134.3	15.0 (8) ± 3.0	-	-
	6	digestion & exercise	45 – 48	4.2	-	0.1343 (8) ± 0.0039	-	3.8 (7) ± 0.0	4.2 (7) ± 0.1	1923.3 (8) ± 110.9	25.0 (7) ± 3.7	-	-

* acclimation temperature/(acute) experimental temperature, ** U_{crit} value of individuals without burst capacity (n = 1) removed from statistical analysis

A4: PUBLICATION IV

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2017

Marine Ecology-Progress Series, 571, 183-191

submitted: 6 June 2016
accepted: 24 March 2017
published: 17 May 2017
doi: 10.3354/meps12130

https://www.int-res.com/abstracts/meps/v571/p183-191/

Vol. 571: 183–191, 2017
https://doi.org/10.3354/meps12130

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published May 17

Impact of ocean warming and acidification on the behaviour of two co-occurring gadid species, *Boreogadus saida* and *Gadus morhua*, from Svalbard

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ABSTRACT: Ocean acidification induces strong behavioural alterations in marine fish as a consequence of acid–base regulatory processes in response to increasing environmental CO_2 partial pressure. While these changes have been investigated in tropical and temperate fish species, nothing is known about behavioural effects on polar species. In particular, fishes of the Arctic Ocean will experience much greater acidification and warming than temperate or tropical species. Also, possible interactions of ocean warming and acidification are still understudied. Here we analysed the combined effects of warming and acidification on behavioural patterns of 2 fish species co-occurring around Svalbard, viz. polar cod *Boreogadus saida* and Atlantic cod *Gadus morhua*. We found a significant temperature effect on the spontaneous activity of *B. saida*, but not of *G. morhua*. Environmental CO_2 did not significantly influence activity of either species. In contrast, behavioural laterality of *B. saida* was affected by CO_2 but not by temperature. Behavioural laterality of *G. morhua* was not affected by temperature or CO_2; however, in this species, a possible temperature dependency of CO_2 effects on relative laterality may have been missed due to sample size restrictions. This study indicates that fish in polar ecosystems may undergo some, albeit less intense, behavioural disturbances under ocean acidification and in combination with ocean warming than observed in tropical species. It further accentuates species-specific differences in vulnerability.

KEY WORDS: Ocean acidification · Climate change · Fish behaviour · Laterality · Activity · Polar habitat · Atlantic cod · Polar cod

INTRODUCTION

Ocean acidification (OA), i.e. the perturbation of seawater carbonate chemistry by accumulating CO_2, has the potential to strongly alter the behaviour of various marine teleosts and elasmobranchs, affecting for example their activity, boldness, predator avoidance, learning and behavioural laterality, and interfering with their sensory processes (Heuer & Grosell 2014). While behavioural alterations have mostly been observed under acute exposure to increased CO_2 partial pressure (pCO_2), there also appears to be a species-specific potential to adapt behaviour across generations (Miller et al. 2012, Allan et al. 2014, Munday et al. 2014, Welch et al. 2014). However, OA develops in parallel to ocean warming (OW), but to date, interactive effects of OA and OW on the behaviour of teleosts remain understudied and have been

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analysed only in a few tropical species (Nowicki et al. 2012, Domenici et al. 2014, Ferrari et al. 2015). In cold-adapted fish species, OA-induced behavioural changes have not been assessed, although the polar ocean of the northern hemisphere is expected to experience the greatest changes in both temperature and pCO_2-induced acidification in the near future (IPCC 2013).

Here we analysed the combined effects of OA (as projected for the year 2100) and temperature on spontaneous activity and behavioural laterality of 2 co-occurring teleost species from Svalbard, Norway. These types of behaviour have been shown to be affected by CO_2 in tropical (activity and laterality) and temperate (laterality) fish species. CO_2-induced effects on activity are species dependent, with activity being either increased or reduced by a predicted rise in CO_2 (Munday et al. 2010, 2013, 2014, Cripps et al. 2011, Nowicki et al. 2012). Behavioural lateralization is defined as the side preference of an animal conducting a certain task (e.g. 'handedness') or, in this study, the tendency to turn to one side at the end of an experimental runway (Domenici et al. 2012). Earlier studies observed a reduction in the behavioural laterality of tropical and temperate fish species after acclimation to OA scenarios (Domenici et al. 2012, 2014, Jutfelt et al. 2013) with the exception of temperate Atlantic cod *Gadus morhua* and the temperate wrasse *Ctenolabrus rupestris* (Jutfelt & Hedgärde 2015, Sundin & Jutfelt 2016).

Increased spontaneous activity can lead to higher energetic demands, requiring more food uptake, which can subsequently lead to greater exposure to predators (Munday et al. 2013). Conversely, an increase in activity could be the consequence of reduced foraging success or increased energetic demand leading to intensified foraging behaviour to fill this energetic gap (Cripps et al. 2011). The potential effects of behavioural lateralization on animal fitness are not fully resolved. Lateralized behaviour reflects functional asymmetry of the brain, where one brain hemisphere specializes in conducting a certain task. Specialization may be useful to increase the speed of sensory processing when several different stimuli must be analysed simultaneously (Rogers et al. 2004). This is especially important in fish, such as Gadidae, that possess lateral eyes and no mobile neck, so that each eye (and thus each brain hemisphere) perceives an almost entirely different set of visual information (Vallortigara & Rogers 2005). Furthermore, fish lack the corpus callosum that accelerates information transfer between brain hemispheres in placental mammals (Dadda et al. 2009). Dadda et al. (2010) found a correlation between the degree of behavioural lateralization and escape performance in teleost prey fish. As a trade-off, non-lateralized animals performed better at cognitive tasks than lateralized fish when relevant similar stimuli occurred simultaneously on both sides of the body (Dadda et al. 2009). While these findings explain why non-lateralized animals are also commonly found in the wild, they complicate prediction of ecological consequences caused by changes in laterality on a population level. Here, we interpret a change in behavioural laterality as a proxy for disturbance in nervous system functioning, in similar ways as reported by Domenici et al. (2012).

The polar cod *Boreogadus saida* has a circumpolar distribution in Arctic and subarctic waters and is considered a key species in the Arctic ecosystem (Hop & Gjøsæter 2013). The Atlantic cod *Gadus morhua* is a temperate fish species which has shifted its distribution farther north with recent warming (Sundby & Nakken 2008, Drinkwater 2009). At present, the distribution areas of *B. saida* and *G. morhua* overlap for most of the year in the coastal waters around Svalbard, where sea surface temperature fluctuates between −1.8°C in winter and up to 8°C in summer (Renaud et al. 2012, Beierlein et al. 2015). The surface water temperature of this area is predicted to increase further by 2.5°C until the year 2100 according to the Representative Concentration Pathway (RCP) 8.5 scenario (IPCC 2013). The consequences of further temperature-driven northward migration of *G. morhua* and its interaction with *B. saida* on the Arctic ecosystem are unknown, especially as simultaneous OA might alter the usual behaviour of each species. We sought to document the species-specific vulnerability of the behaviour of both *B. saida* and *G. morhua* in response to combined OW and OA. We therefore incubated *B. saida* and *G. morhua* for 6 wk under present-day and future pCO_2, with the latter being set close to the maximum pCO_2 value projected by RCP 8.5 for the year 2100 (IPCC 2013). Animals were incubated at 4 different temperatures, between 0 and 8°C for *B. saida* and between 3 and 16°C for *G. morhua*, to cover a broad overlapping range of temperatures from the thermal window of each species.

MATERIALS AND METHODS

Animal collection

Juvenile *Boreogadus saida* were caught at 120 m depth in the inner part of the Kongsfjord on a polar night trawl on 17 January 2013 (78.97° N, 12.51° E).

Subsequently, the animals were kept in facilities of the Tromsø Aquaculture Research Station, in Kårvik, Norway. Juvenile *Gadus morhua* were caught in various locations of western Svalbard between 26 and 29 August 2013 on a cruise of the RV 'Heincke' in Rijpfjorden (80.15° N, 22.12° E), Hinlopenstretet (79.30° N, 18.57° E) and Forlandsundet (78.54° N, 11.3° E). A fish lift combined with a pelagic mid-water trawl was used to catch the animals (Holst & McDonald 2000). Further information on the cruise is available at http://doi.pangaea.de/10.1594/PANGAEA.824703. Specimens of both species were transported to the Alfred Wegener Institute in Bremerhaven, Germany, and kept in aquaria at a water temperature of 5°C in a recirculating seawater system prior to the start of the incubation.

Incubation

Experiments on *B. saida* and *G. morhua* started in June 2013 and June 2014, respectively. *B. saida* and *G. morhua* were incubated at 0, 3, 6 and 8°C and at 3, 8, 12 and 16°C, respectively. pCO_2 was either 374–515 µatm (control CO_2) or 852–1416 µatm (high CO_2) in a full factorial approach with a group size of 12 animals treatment^{-1}, resulting in a sample size of 96 animals for each species. Animals were transferred into individual tanks (height: 35 cm, diameter: 30 cm, volume: ~24 l with a flow-through of ~500 ml min^{-1}) and randomly distributed among treatment groups. The animals were kept separately in order to enable quantification of feed consumption of each individual, which was published separately (Kunz et al. 2016). Water supply occurred through a re-circulating aquarium system with a total volume of 10 m^3. The seawater for the system was collected in 'Tiefe Rinne', close to Heligoland (Helgoland), Germany, in the North Sea. Adequate water quality was ensured through nitrification filters, UV-sterilizers and protein skimmers, and the nitrate concentration was kept at <50 mg l^{-1} at all times. Temperature was adjusted in 4 temperature-controlled rooms (1 room for each temperature treatment) by a maximum change of 2°C d^{-1} for each group, starting from 5°C. pCO_2 in high-CO_2 groups was increased within 1 d after the temperatures were adjusted. The incubation period started after the desired temperature and CO_2 condition had been reached for each treatment group. The animals were fed ad libitum with a commercial pellet food (Amber Neptun, Skretting) every fourth day. Day/night cycle was 12:12 h, with lights on at 08:00 h. Oxygen concentration in fish tanks was measured occasionally throughout the incubation period and was always found to be ~100%. Apart from temperature, room conditions were kept as similar as possible, with similarly dimmed light and a small distance between shelves containing the tanks with different CO_2-treatments (~1 m). Opaque walls of the tanks shielded external stimuli effectively, and activities inside the rooms were kept to a minimum. Behavioural experiments were conducted 6 wk after onset of the incubation and lasted 8 d in total. Length and weight of each animal were measured at the beginning of the incubation and 1 d after the end of the behavioural experiments. Mean lengths and weights (±SD) of individuals in each treatment group and species are available in Tables S1 & S2 in the Supplement at www.int-res.com/articles/suppl/m571p183_supp.pdf. One out of 96 *B. saida* and 8 out of 96 *G. morhua* died during the incubation period for unknown reasons. The 8 casualties among *G. morhua* occurred in 5 different treatment groups at all temperatures as well as at control and high pCO_2, with no more than 2 specimens dying per treatment group. These mortalities were thus considered independent of the treatment conditions. A representative image of the incubation system of one treatment group is provided in Fig. S1 in the Supplement.

CO_2 and carbonate chemistry

Seawater was aerated with an air/CO_2 mixture from a gas-mixing pump (HTK) before flowing into the tanks holding the animals. Temperature, salinity, dissolved inorganic carbon and pH_{tot} were determined at least once weekly in order to calculate the seawater carbonate parameters. Means were calculated for each week; Tables S1 & S2 list the means ± SD over the whole incubation period for each treatment group and species. Detailed methodological information and the raw data are provided at https://doi.pangaea.de/10.1594/PANGAEA.866369.

Behavioural testing

Spontaneous activity

Spontaneous activity was tested 2 d after feeding. A camera was installed in the centre above the housing tank of an animal next to a white LED lamp for better illumination. Recordings were started manually 10 min after camera installation and illumination. The recordings lasted for at least 10 min, and the last

5 min were used for quantification of spontaneous activity. For post-processing of the video, a grid was placed centrally over the tank, dividing it into 4 equally sized rectangles using the software packages ImageJ and Dartfish®. The frequency of grid lines crossed was counted for each individual within a 5 min period of recording. A crossing was counted when the whole head of an animal crossed a grid line (ending right before the pectoral fins). For each animal, the total number of grid lines crossed was divided by 5 to obtain the number of lines crossed min^{-1}. Operator-controlled analysis of behaviour was performed in a randomized order for each species without knowing animal or treatment to avoid any observer bias. Videos were recorded throughout the whole day, whereby only animals of one temperature treatment were observed per day. The sequence of video recordings alternated between the 2 CO_2 treatments to compensate for possible daytime-related differences in activity. In total, data from 94 *B. saida* and 87 *G. morhua* were used to quantify spontaneous activity. Video recordings for 2 animals had to be discarded for technical reasons.

Behavioural laterality

On the same day, after recording activity, each fish was transferred into a 125 × 50 cm aquarium containing a 2-sided T-maze, similar to the maze used by Domenici et al. (2012), to investigate combined effects of temperature and CO_2 on behavioural laterality via a detour test. The opaque maze, with a runway length of 70 cm and width of 8 cm, was placed in the centre of the aquarium. Perpendicular to each of the maze's ends was a dark grey, opaque barrier with a length of 25 cm leaving a gap of 5.5 cm on each side so that the animal could leave the maze on either left or right (see Fig. S2 in the Supplement for a scheme of the setup). The sides of the aquarium were shielded with a dark grey cover. The aquarium was filled with 10 cm of seawater according to the test animal's treatment conditions. After an acclimation period of 10 min, the animal was gently encouraged to swim through the maze by approaching it from behind with a meshed plastic slide until the animal reached the end of the runway where it escaped to the left or to the right. The side on which the individual left the maze was noted. This procedure was repeated 14 times for each fish, whereby the swimming direction through the chamber was reversed after each trial to compensate for the potentially disturbing influence of the fish's orientation towards existing room-related structures. Absolute and relative laterality indices were calculated as described by Domenici et al. (2012). Absolute laterality quantifies the preference of an animal for one side over the other; thus an animal that turned to the same side every time was allocated an absolute laterality index of 100. In contrast, the relative laterality index takes the side preference of each animal into account. An animal that turned to the left every time was allocated a relative laterality index of −100 and an animal that turned to the right every time was assigned a relative laterality index of +100. All trials were conducted by the same experimenter and lasted about 10 min for each animal. In total, data from 95 *B. saida* and 88 *G. morhua* were tested for behavioural laterality.

Statistical analysis

Spontaneous activity and absolute and relative laterality were analysed by an ordinary 2-way ANOVA to test for significant effects of temperature, CO_2 and possible interactions of these 2 factors. Normality of each group was investigated via D'Agostino and Pearson omnibus normality tests and the homogeneity of variances via a Brown-Forsythe test with $\alpha = 0.05$. A significant deviation from a normal distribution was detected in 3 out of 48 groups tested (*B. saida*: spontaneous activity at 8°C and high pCO_2; absolute laterality at 6°C and low pCO_2. *G. morhua:* spontaneous activity at 8°C and high pCO_2). However, using an α of 0.05 sets the chance of a false positive Type 1 error of each normality test to 5%, which may account for the deviation from normality in those 3 out of 48 tested groups. Furthermore, in 2 out of 3 cases, the observed violation of normality was caused by a single animal, and an exclusion of these animals did not lead to disappearance of the observed significant findings. We thus concluded that it is still acceptable to use the 2-way ANOVA under these conditions. A coefficient of variation (CV) was determined for each treatment group of spontaneous activity data by calculating the ratio of standard deviation and mean values, and the difference between the 2 species was analysed using a 2-sided Mann-Whitney test ($\alpha = 0.05$). Correlation between animal length and spontaneous activity was tested with a 2-tailed non-parametric Spearman r-test. The CO_2 effect on side preference on a population level and a possible CO_2-induced change from a non-random to a random distribution of left and right turns were tested for each species by pooling relative laterality data of all temperatures in accordance to control or high pCO_2, as we had not detected a significant

temperature effect on behavioural laterality in either *B. saida* or *G. morhua*. Subsequently, 2-sided 1-sample *t*-tests were conducted ($\alpha = 0.05$) for each CO_2 treatment of each species testing for significant differences from the hypothetical mean of 0. Deviation from a random binomial distribution was tested for via a log likelihood ratio goodness of fit test (*G*-test) using the software 'R' (v. 3.2.3) and the R-package 'DescTools'. GraphPad Prism® 6 was used for all other statistical tests and for generation of figures.

RESULTS

Spontaneous activity

Spontaneous activity of *Boreogadus saida* increased significantly with rising ambient temperature between 0 and 8°C ($p < 0.001$, $F_{3,86} = 7.064$, Fig. 1A). No significant difference in spontaneous activity of *B. saida* was detected between control and high CO_2 concentrations ($p = 0.0700$, $F_{1,86} = 3.368$). In contrast, spontaneous activity of *Gadus morhua* did not significantly depend on temperature ($p = 0.3172$, $F_{3,79} = 1.195$, Fig. 1B) or CO_2 concentration ($p = 0.5024$, $F_{1,79} = 0.4540$). *G. morhua* displayed a non-significant trend towards greater mean activity with increasing temperature which was strong between 3 and 8° but levelled off at higher temperature and even decreased in the group at 16°C under normal CO_2 levels. A significant interaction between temperature and CO_2-related effects was not detected in either species (all $p > 0.05$). The CV of spontaneous activity of *G. morhua* was significantly higher than the CV of *B. saida* ($p < 0.001$, Fig. 1C). Spontaneous activity was not significantly correlated with body length in either species ($p > 0.05$).

Behavioural laterality

Absolute laterality of *B. saida* was significantly reduced by CO_2 ($p < 0.01$, $F_{1,87} = 7.152$, Fig. 2A), but was not affected by temperature ($p = 0.2156$, $F_{3,87} = 1.518$). Also, in this species, relative laterality was dependent on CO_2 ($p < 0.01$, $F_{1,87} = 10.26$, Fig. 2C), but not on temperature ($p = 0.7020$, $F_{3,87} = 0.4728$), with a shift from left to right orientation under increased CO_2 concentrations. Side preference of *B. saida* was significantly left biased under control CO_2 ($p < 0.05$, $t = 2.242$, df = 47), significantly right biased under high CO_2 ($p < 0.05$, $t = 2.260$, df = 46) and significantly differed from a random binomial distribution under both CO_2 conditions ($p < 0.001$, $G = 13.761$, χ^2 df = 1 for low pCO_2 and $p < 0.01$, $G = 7.0399$, χ^2 df = 1 for high pCO_2).

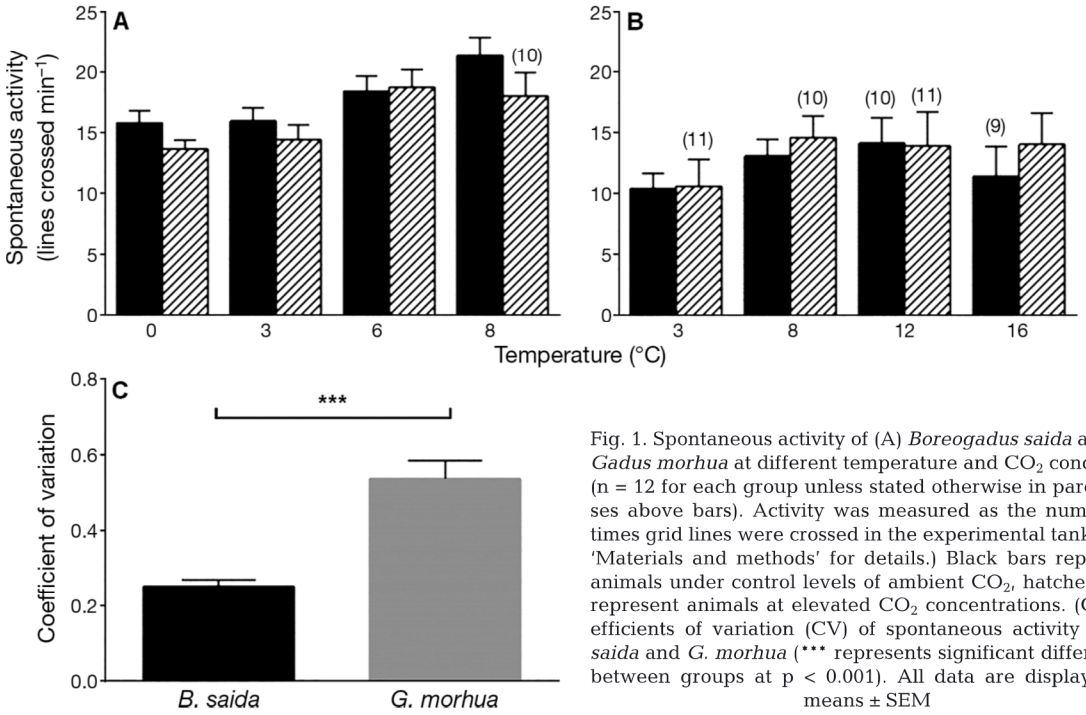

Fig. 1. Spontaneous activity of (A) *Boreogadus saida* and (B) *Gadus morhua* at different temperature and CO_2 conditions (n = 12 for each group unless stated otherwise in parentheses above bars). Activity was measured as the number of times grid lines were crossed in the experimental tanks (see 'Materials and methods' for details.) Black bars represent animals under control levels of ambient CO_2, hatched bars represent animals at elevated CO_2 concentrations. (C) Coefficients of variation (CV) of spontaneous activity for *B. saida* and *G. morhua* (*** represents significant differences between groups at $p < 0.001$). All data are displayed as means ± SEM

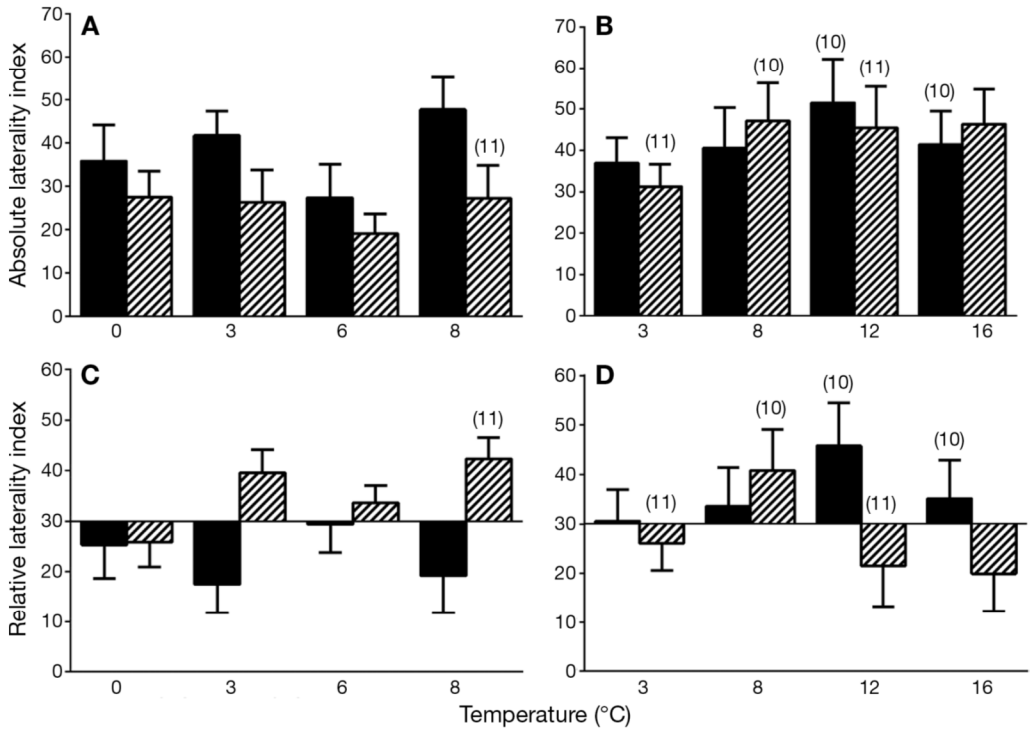

Fig. 2. Absolute laterality index of (A) *Boreogadus saida* and (B) *Gadus morhua* and relative laterality index of (C) *B. saida* and (D) *G. morhua* at different temperature and CO_2 conditions (n = 12 for each group unless stated otherwise in parentheses above bars). Details of laterality indices are given in the 'Materials and methods'. Black bars represent animals under control; hatched bars represent animals at elevated CO_2 concentrations. Data are displayed as means ± SEM

In *G. morhua*, absolute laterality was not affected by CO_2 ($p = 0.9949$, $F_{1,80} = 4.086 \times 10^{-5}$, Fig. 2B) or temperature ($p = 0.3966$, $F_{3,80} = 1.002$). Relative laterality also did not significantly depend on CO_2 or temperature ($p = 0.0913$, $F_{1,80} = 2.920$ and $p = 0.5375$, $F_{3,80} = 0.7293$, respectively, Fig. 2D). *G. morhua* did not exhibit a significant side preference under control or under high CO_2 conditions ($p = 0.1272$, $t = 1.556$, df = 43 and $p = 0.3792$, $t = 0.8886$, df = 43, respectively). The side preference differed significantly from a binomial random distribution under low, but not quite under high CO_2 conditions ($p < 0.01$, $G = 8.4349$, χ^2 df = 1 and $p = 0.09$, $G = 2.8659$, χ^2 df = 1, respectively). In both species, no interactive effects were detected between CO_2 and temperature effects on absolute and relative laterality (all $p > 0.05$).

DISCUSSION

This is the first study analyzing and comparing the combined effects of CO_2 and temperature on the behaviour of 2 gadid fish species, one polar and cold adapted (*Boreogadus saida*), the other (*Gadus morhua*) temperate and invasive to the high polar environments due to global warming. We demonstrated that the behavioural vulnerability of fish, even if they are related, may be species-dependent in response to temperature and CO_2.

While we observed a significant influence of temperature on spontaneous activity of *B. saida*, no such significance was detected in *G. morhua*. However, in the latter, a possible temperature effect may have been masked by high inter-individual variability, which was significantly more pronounced in *G. morhua* than in *B. saida*. We found no CO_2-related effect on the spontaneous activity of *B. saida* and *G. morhua*, in contrast to strong alterations that were observed in tropical cardinalfish (*Apogon cyanosoma* and *Cheilodipterus quinquelineatus*) and damselfish (*Pomacentrus wardi*) (Munday et al. 2010, 2014), which either showed an increase or a decrease in activity in response to elevated pCO_2 as predicted for future OA scenarios. However, CO_2-effects on swimming behaviour of fish appear to be strongly species-dependent, and our results are consistent with findings of several

studies that observed largely resilient routine swimming activity and kinematics particularly in temperate species (including *G. morhua* larvae) (Maneja et al. 2013, 2015, Sundin & Jutfelt 2016), but also in tropical species (Nowicki et al. 2012, Bignami et al. 2013, 2014). As our study was conducted with juvenile specimens of *G. morhua*, it can be concluded that swimming behaviour at least in this species appears to be robust to an increase in environmental CO_2 across different life stages.

Interestingly, the effect of CO_2 on laterality was different in *B. saida* and *G. morhua*. In *B. saida,* absolute lateralization was significantly reduced and paralleled by a shift from left to right lateralization, whereas in *G. morhua*, we found no changes in absolute lateralization or side preference. These results conform with recent experiments on temperate fish species, that found a CO_2-induced reduction of absolute laterality in three-spined stickleback *Gasterosteus aculeatus* (Jutfelt et al. 2013, Lai et al. 2015), but not in wrasse *Ctenolabrus rupestris* or in juvenile *G. morhua* of similar age compared to the specimens in our study (Jutfelt & Hedgärde 2015, Sundin & Jutfelt 2016). Contrary to findings in *Pomacentrus wardi* (Domenici et al. 2014), we did not detect any interaction of temperature- and CO_2-related effects on behavioural laterality. However, potential interactive effects of CO_2 and temperature on the relative laterality of *G. morhua* may have been missed because of low statistical power resulting from a relatively small sample size. At 3, 12 and 16°C, there was a CO_2-induced trend from right to left lateralization in *G. morhua*, with the opposite at 8°C, and we suggest this to be the main reason why the turning directions of *G. morhua* were not significantly different from a random binomial distribution at high CO_2. Inter-individual variability of behavioural lateralization is by definition very high, and one must thus be quite cautious with interpretation of these findings. Based on our results, the possibility of interactive effects on the behaviour of *G. morhua* should not be strictly ruled out. Furthermore, for changes in absolute and relative laterality, high inter-individual variability and low effect sizes may have given rise to potential type I errors, which must be considered when comparing differences in CO_2 effects between *B. saida* and *G. morhua*. A definitive answer to these issues requires further experimental investigation.

Domenici et al. (2014) found a CO_2-induced shift in turning preference from right to left, which was interpreted as a change in task processing from the left to the right brain hemisphere. Across taxa, the right brain hemisphere is associated with stress-related endocrine responses and reactive behavioural patterns (Rogers 2010). In humans, the right brain hemisphere is the predominant driver of the pituitary–adrenal axis and of sympathetic cardiac control (Wittling & Pflüger 1990, Wittling et al. 1998). In contrast, the left brain hemisphere is associated with the execution of routine behaviour (Rogers 2010). A shift in laterality from right to left preference under future OA scenarios as observed by Domenici et al. (2014) would thus indicate a shift in the stress-related cognitive state of the fish, i.e. a CO_2-induced shift from a low to a high stress level. Those explanations (inverted function or changing stress level) may not be mutually exclusive, as Hamilton et al. (2014) found a CO_2-induced increase of anxiety in rockfish, which could be an indication for a shift to a more active right brain hemisphere. In the study of Hamilton et al. (2014), the mentioned increase in anxiety was related to altered $GABA_A$-receptor functioning. Speculatively bringing these hypotheses together, an inversion of the $GABA_A$-receptor function could also be the cause of a shift in brain hemispherical usage which could then be responsible for the shift in side preference.

The question arises why the shift in side preference was opposite in *B. saida*. Hemispheric laterality is generated during ontogenesis and can be inversed, as shown in domestic chicken by Rogers (1990). This could also be the case for *B. saida*; thus, our findings might still have the same implications as in coral reef fishes. However, this explanation remains speculative, and its verification requires further exploration. The CO_2-induced reduction in absolute lateralization of *B. saida* may indicate reduced fitness under future OA scenarios, as the degree of lateralization may correlate with other behavioural parameters such as efficiency of predator avoidance (Dadda et al. 2010). However, predictions about the ecological consequences of our findings need to be made with care, as the animals in our study were kept separate from each other. Furthermore, the sudden availability of more space during laterality tests may have had an unknown effect on the observed outcome. It cannot be excluded that the fish may have behaved differently if they had been incubated under more natural conditions in schooling groups with social hierarchies. Nevertheless, both species were treated similarly and thus comparison of temperature and CO_2 effects between these species remains meaningful.

Overall, elevated CO_2 levels may affect some behavioural patterns of cold-adapted teleosts, but our findings also indicate species-specific differences in behavioural resilience to OA. Our results are similar

to those obtained in 3 other studies on temperate fish species. A significant CO_2 effect on behaviour, including behavioural laterality and activity, was found in three-spined stickleback, but again, not in temperate Atlantic cod, indicating a reduced vulnerability of behaviour in this species to an increase in environmental CO_2 (Jutfelt et al. 2013, Jutfelt & Hedgärde 2013, 2015). This may be an adaptive trait reflecting its demersal mode of life and repeated exposure to hypoxia and hypercapnic water layers (Neuenfeldt et al. 2009). Due to preadaptation to different environments and levels of variability, the degree of alterations of behaviour under increased pCO_2 may vary strongly between species. The mechanisms causing the disturbance of behaviours may include accumulation of bicarbonate in the body fluids (Nilsson et al. 2012) which results from acid–base regulation compensating for CO_2-induced acidification (Ishimatsu et al. 2008). The physiological systems supporting behaviour to be insensitive to elevated CO_2 (and possibly, bicarbonate accumulation) remain to be investigated. Such understanding will be crucial for projecting teleost resilience under future CO_2 scenarios (Wittmann & Pörtner 2013). As discussed above, it may be possible that CO_2 effects on the behaviour of *G. morhua* are dependent on the environmental temperature. This would make Atlantic cod a useful species for elaboration of the physiological mechanisms determining behavioural vulnerability or resistance of fish species in a future, more acidified ocean.

In summary, this study indicates that the behaviour of *B. saida* is more vulnerable to future OA than the behaviour of *G. morhua*. We did not observe significant temperature-driven modulation in the extent of behavioural alteration; however, in *G. morhua*, interactive effects of temperature and CO_2 might have been missed due to the small size of treatment groups. Nevertheless, the temperature-independent reduction in the behavioural laterality of *B. saida* may indicate reduced fitness of this species in a high CO_2 world, which might place it at a disadvantage in competitive and predator–prey interactions with *G. morhua* in the waters around Svalbard. Future warming of the area can lead to an increasing population size of *G. morhua* and a further northward shift of species distribution areas (Perry et al. 2005). In a warmer, more acidified, open ocean, *G. morhua* may outcompete *B. saida* in the long term. However, the potential of species to acclimate or adapt their behaviour under combined OA and OW over generations has received little attention (Allan et al. 2014) and urgently demands further investigation.

Acknowledgements. We thank Jasmine Nahrgang for providing the specimens of *B. saida* (funded by the Polarisation grant of the Norwegian Research Council, no. 214184/F20), Janina Popp for her contribution to data analysis and Philip Munday for helpful suggestions for the experimental design. Silvia Hardenberg, Sebastian Berger and Guido Krieten helped with the technical realization of the incubation setup and during the incubation period. We also thank Karim Zanaty, Marcel Machnik, Benjamin Matthei, Fredy Vèliz Moraleda, Anette Tillmann, Isabel Ketelsen and Timo Hirse for their contributions to the measurements of pH and dissolved inorganic carbon. We appreciate the helpful statistical advice of Stephan Frickenhaus and thank Nils Koschnick and Heidrun Windisch for various services they provided throughout the incubation period. This work was funded by the Federal Ministry of Education and Research (BMBF, no. FKZ 03F0655B), Germany. The experiments were conducted in accordance with the ethical standards of the federal state of Bremen, Germany, and were approved under reference number 522-27-11/02-00 (93). The tested animals were used in subsequent experiments that will be published elsewhere and were ultimately sacrificed for organ removal.

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Editorial responsibility: Alejandro Gallego, Aberdeen, UK

Submitted: June 6, 2016; Accepted: March 24, 2017
Proofs received from author(s): April 20, 2017

A5: PUBLICATION V

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2017

Frontiers in Zoology, 14(1), 1–12

submitted: 14 February 2017
accepted: 30 March 2017
published: 14 April 2017
doi: 10.1186/s12983-017-0205-1

https://frontiersinzoology.biomedcentral.com/articles/10.1186/s12983-017-0205-1

Leo *et al. Frontiers in Zoology* (2017) 14:21
DOI 10.1186/s12983-017-0205-1

Frontiers in Zoology

RESEARCH Open Access

Mitochondrial acclimation potential to ocean acidification and warming of Polar cod (*Boreogadus saida*) and Atlantic cod (*Gadus morhua*)

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Abstract

Background: Ocean acidification and warming are happening fast in the Arctic but little is known about the effects of ocean acidification and warming on the physiological performance and survival of Arctic fish.

Results: In this study we investigated the metabolic background of performance through analyses of cardiac mitochondrial function in response to control and elevated water temperatures and PCO_2 of two gadoid fish species, Polar cod (*Boreogadus saida*), an endemic Arctic species, and Atlantic cod (*Gadus morhua*), which is a temperate to cold eurytherm and currently expanding into Arctic waters in the wake of ocean warming. We studied their responses to the above-mentioned drivers and their acclimation potential through analysing the cardiac mitochondrial function in permeabilised cardiac muscle fibres after 4 months of incubation at different temperatures (Polar cod: 0, 3, 6, 8 °C and Atlantic cod: 3, 8, 12, 16 °C), combined with exposure to present (400μatm) and year 2100 (1170μatm) levels of CO_2.
OXPHOS, proton leak and ATP production efficiency in Polar cod were similar in the groups acclimated at 400μatm and 1170μatm of CO_2, while incubation at 8 °C evoked increased proton leak resulting in decreased ATP production efficiency and decreased Complex IV capacity. In contrast, OXPHOS of Atlantic cod increased with temperature without compromising the ATP production efficiency, whereas the combination of high temperature and high PCO_2 depressed OXPHOS and ATP production efficiency.

Conclusions: Polar cod mitochondrial efficiency decreased at 8 °C while Atlantic cod mitochondria were more resilient to elevated temperature; however, this resilience was constrained by high PCO_2. In line with its lower habitat temperature and higher degree of stenothermy, Polar cod has a lower acclimation potential to warming than Atlantic cod.

Keywords: Arctic fish, RCP 8.5, Heart mitochondria, Mitochondrial capacity, Proton leak

Background

Ocean warming driven by anthropogenic CO_2 emissions influences the distribution of marine animals causing significant impacts on biodiversity and ecosystem structure [1, 2], such as local extinctions [3] and poleward migrations [4–6]. Fish (and other ectotherms) are particularly sensitive to fluctuations in temperature since their body temperature is in equilibrium with their environmental temperature [7]. Fish species distribution, in fact, is confined to a specific temperature window, due to the temperature dependency of physiological processes and to sustain maximal energy efficiency ([8] for review).

The increased CO_2 concentration in the atmosphere is one of the major causes for the global greenhouse effect and also causes a decrease in ocean pH, a phenomenon commonly known as ocean acidification [9]. High CO_2 partial pressure (PCO_2) is known to affect biological and physiological processes of marine organisms (e.g. [10–14]) and tolerances towards other stressors [15–17]. Moreover, high PCO_2 could provoke a narrowing of the thermal

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tolerance window of ectotherms, so that limits of its thermal acclimation capacity are met earlier [2, 18–21].

At the cellular level, exposure to high temperature can cause changes in the three dimensional structures of proteins, including the assembly states of multiprotein complexes and eventually protein denaturation and loss of activity [7]. Moreover, increasing temperatures can alter the cellular membranes packing order, which can cause changes in membrane-associated processes until a potential complete loss of function [22]. Furthermore, since cellular oxygen demand increases with increasing temperature, the production of mitochondrial reactive oxygen species (ROS) is likely to increase which can damage biological molecules, including lipids, proteins and DNA [23, 24]. Therefore, towards the upper limit of the thermal window, the cellular energetic costs for maintenance rise, increasing baseline energy turnover and allowing only for time-limited periods of passive tolerance. If high temperature persists over this period of passive tolerance, the costs of maintenance can only be covered at the expense of other functions such as growth and reproduction, decreasing the overall animal fitness [17]. Therefore, in light of ongoing ocean acidification and warming it is important to understand how fish respond to increasing habitat temperatures, their ability to adjust their thermal sensitivity and the role that high PCO_2 plays in thermal acclimation [2, 25].

The fish heart is highly aerobic and sensitive to temperature [26, 27]. Its capacity limits have been hypothesized to shape the warming-induced onset of sublethal thermal constraints in fishes [2, 28–31]. Recent studies have shown that high temperature leads to heart failure in various fish species like New Zealand triplefins and temperate and tropical wrasses [28, 29, 32, 33]. It was suggested that progressive impairment of several components of the mitochondrial function measured in permeabilised heart muscle fibres, such as oxidative phosphorylation (OXPHOS, respiratory state III), ATP production efficiency and the capacity of single complexes of the Electron Transport System (ETS) shape the temperature of heart failure (T_{HF}). High temperature changes the fluidity of mitochondrial membranes, which can entail increased proton leak through the inner membrane ([19] for review), resulting in decreased coupling ratios and causing decreased membrane potential [34, 35] and, as a consequence, inhibit the electrogenic transport of substrates, i. e. the transport of charged substrates like glutamate and malate that leads to the translocation of net charge across the membrane [36]. This indicates that mitochondrial metabolism is involved in functional constraints and thermal limitation of this tissue [28, 29, 32, 33]. Therefore, alterations in cardiac mitochondrial metabolism might lead to impaired cardiac energy turnover and, as a consequence, constraints in cardiac performance and ultimately affect the fishes' thermal sensitivity.

Although an extensive literature has been produced on the effects of temperature on fish cellular metabolism and mitochondrial function (e.g. [8, 33, 37] and the literature therein), only few studies have addressed the effects of moderately elevated PCO_2 on them [30, 38–41]. Moreover, as ocean warming and ocean acidification caused by high PCO_2 are two sides of the same coin, they must be considered in combination in order to draw ecologically realistic conclusions [17, 42, 43].

Ocean acidification and warming trends are projected to exert particularly strong effects in the Arctic. As one of the consequences, temperate species may become established in Arctic habitats (by poleward migration), potentially displacing resident taxa [1, 4, 6]. For example, in the past decade the Northeast Arctic population of Atlantic cod (*Gadus morhua,* NEAC) has expanded its range into the Barents Sea [44, 45], on the North-east Greenland shelf [46] and in the coastal waters around Svalbard, which are inhabited by native Polar cod (*Boreogadus saida*), a key species in this region [1, 47].

Polar cod is a permanently cold adapted Arctic fish (thermal habitat around Svalbard ranging from −2 to +7 °C [48, 49]) while NEAC is a cold acclimated sub-Arctic population of temperate Atlantic cod expanding into the Arctic (habitat thermal range around Svalbard: 0–8 °C [1, 50]). Cold-acclimated and -adapted fish are known to have elevated mitochondrial densities. Among cold adapted species, extreme stenotherms such as high Antarctic fish, have high densities but low mitochondrial capacities and low proton leak in aerobic tissues [37, 51–53]. This may result in the low maintenance costs derived by proton leak and narrow thermal windows of these species and, as a consequence, cause high sensitivity to ocean warming [53, 54]. On the other hand, eurythermal cold adaptation ensures mitochondrial function over a wider range of temperatures at lower mitochondrial densities and maximized capacities [53, 55]. As a permanently cold adapted fish, Polar cod may therefore not be able to adjust mitochondrial capacities during warming to a similar extent as NEAC, which apparently has a higher capacity to adjust to higher temperatures by decreasing mitochondrial densities and capacities and thereby developing the metabolic plasticity necessary to acclimate to new conditions [56]. The differences in thermal response and, in particular, the ability to acclimate to higher temperatures will play a central role for their interaction in a changing ecosystem.

Hence, the aim of this study was to investigate the acclimation potential of Polar cod *Boreogadus saida* and Northeast Arctic cod (NEAC) *Gadus morhua* exposed to water temperatures and PCO_2 projected for

the year 2100 in the Arctic i.e. 8 °C and 1170μatm PCO_2 (RCP 8.5 [57]). For a deeper understanding of the impact of ocean acidification and warming on the bioenergetics of the two species in relation to thermal tolerance, we further investigated mitochondrial function in the cardiac muscle of animals incubated for 4 months at four different temperatures (Polar cod: 0, 3, 6, 8 °C and Atlantic cod: 3, 8, 12, 16 °C), and two PCO_2 (400μatm and 1170μatm) in a cross factorial design. We used permeabilised cardiac muscle fibres to investigate a system resembling the living state as closely as possible [58–60], facilitating the extrapolation from measurements of cardiac mitochondrial capacities to their potential effects on the heart and eventually drawing conclusions on the effects of high temperature and high PCO_2 on the whole organism. Moreover, by analysing the mitochondrial function at the respective incubation temperature we could investigate the acclimation potential of the two species. We hypothesized that NEAC had higher thermal limits and a larger acclimation capacity than Polar cod and found accordingly that mitochondrial functions are constrained at lower temperatures in Polar cod than in NEAC. We discuss our results in light of the findings reported by Kunz et al. [61], who showed wider thermal windows for growth and standard metabolic rate (SMR) in NEAC than in Polar cod from the same acclimation experiment.

Methods

Animal collection

Juvenile Polar cod were collected by bottom trawl in combination with a fish lift [62] on January 17th, 2013 from the inner part of Kongsfjorden (Svalbard, 78° 97' N 12°51' E) at 120 m depth and a water temperature between 2 and 3 °C. They were kept at 3.3–3.8 °C in the facilities of the Tromsø Aquaculture Research Station, in Kårvik (Norway) until late April 2013 when they were transported to the aquarium facilities of the Alfred Wegener Institute (AWI) in Bremerhaven (Germany), where they were kept at 5 °C, 32 PSU and ambient PCO_2 until the start of the incubation.

Juvenile Northeast Arctic cod (NEAC) were caught in late August 2013 in several locations off Western Svalbard during RV Heincke cruise HE408 in Rijpfjorden (80° 15.42' N 22° 12.89' E), Hinlopenstretet (79° 30.19' N 18° 57.51' E), and Forlandsundet (78° 54.60' N 11° 3.66' E) at 0–40 m depth and water temperatures between 3.5 and 5.5 °C using a pelagic midwater trawl in combination with a fish lift [62]. The specimens were transported to the AWI facilities in Bremerhaven (Germany), where they were kept at 5 °C, 32 PSU and ambient PCO_2 until the start of the incubation.

Incubation

Polar cod incubation started in June 2013 and of NEAC in May 2014. After at least 4 weeks of acclimation to laboratory conditions (5 °C, 32 PSU and ambient PCO_2), individuals from both species were housed in single tanks and randomly allocated to the temperature and PCO_2 incubation set-up with a 12 h day/night rhythm. The respective PCO_2 conditions were pre-adjusted in a header tank containing ~200 l of seawater. Virtually CO_2-free pressurized air and pure CO_2 were mixed by means of mass flow controllers (4 and 6 channel MFC system, HTK, Hamburg, Germany) to achieve the desired PCO_2. Temperature was adjusted by 1 °C per day for each group starting from 5 °C. PCO_2 in the high PCO_2 group was adjusted within 1 day after the incubation temperature was reached. The animals were kept under incubation conditions for 4 months and fed *ad libitum* with commercial pellet feed (Amber Neptun, 5 mm, Skretting AS, Norway) every fourth day [61]. The sampling of Polar cod and NEAC took place after 4 days of fasting, due to sampling and experimental logistics three to six individuals of Polar cod and four to eight individuals of NEAC were sampled in one batch. Because of a failure in the power supply the group incubated at 3 °C and high PCO_2 died before the mitochondrial capacity could be investigated.

Average length and weight, as well as the number of the specimens per treatment at the time of sampling are given in Table 1.

CO_2 and carbonate chemistry

Temperature, salinity, DIC and pH (total scale) were measured once to twice a week in triplicates in order to monitor the seawater chemistry of the incubation. Temperature and salinity were measured with a WTW LF 197 multimeter (WTW, Weilheim, Germany). pH was measured with a pH meter (pH 3310, WTW, Weilheim, Germany) calibrated with thermally equilibrated NBS-buffers (2-point-calibration). The pH-values were then corrected to pH Total scale using pH-defined Tris-Buffer (Batch 4, Marine Physical Laboratory, University of California, San Diego, CA, USA).

DIC was measured by a Seal QuAAtro SFA Analyzer (800 TM, Seal Analytical, Mequon, United States of America). Calculations of the carbonate system were conducted using CO2sys [63], applying the K1, K2 constants after Mehrbach et al. [64], refitted after Dickson and Millero [65] and using KHSO4 dissociation constants after Dickson [66] assuming a pressure of 10 dbar.

Complete summaries of the seawater parameters and raw data for both species are available from the Open Access library PANGAEA [67, 68].

Table 1 Total length, body weight and number of fish (*n*) used for testing cardiac mitochondrial respiration in Polar cod (*B. saida*) and NEAC (*G. morhua*)

Acclimation	Species					
	B. saida			*G. morhua*		
	Total length (cm)	Body weight (g)	*n*	Total length (cm)	Body weight (g)	*n*
0 °C control	15.28 ± 0.37	22.88 ± 2.05	5	-	-	-
0 °C high	14.30 ± 0.64	19.22 ± 2.61	6	-	-	-
3 °C control	15.62 ± 0.98	27.16 ± 6.25	3	20.04 ± 0.92	60.84 ± 9.81	5
3 °C high	-	-	-	21.61 ± 0.46	78.19 ± 6.91	8
6 °C control	15.73 ± 0.21	25.21 ± 1.14	6	-	-	-
6 °C high	17.52 ± 0.61	32.17 ± 2.90	5	-	-	-
8 °C control	15.18 ± 0.72	20.52 ± 2.56	6	23.26 ± 1.75	99.04 ± 22.13	5
8 °C high	15.07 ± 0.47	18.76 ± 1.11	4	21.51 ± 0.82	80.51 ± 10.46	8
12 °C control	-	-	-	22.70 ± 0.80	98.70 ± 13.14	6
12 °C high	-	-	-	23.42 ± 0.72	100.75 ± 9.22	8
16 °C control	-	-	-	21.56 ± 0.69	81.48 ± 9.37	4
16 °C high	-	-	-	24.27 ± 1.91	133.13 ± 31.87	6

"control" and "high" indicate control (400μatm) and high (1170μatm) CO_2 concentrations. Values are given as means ± S.E.M

Preparation of permeabilised cardiac fibres

Fish were anaesthetized with 0.2 g l^{-1} tricaine methane sulphonate (MS222) and killed by a spinal cut behind the head plate. Hearts were rapidly excised and washed with ice-cold modified relaxing buffer BIOPS (2.77 mM CaK_2EGTA, 7.23 mM K_2EGTA, 5.77 mM Na_2ATP, 6.56 mM $MgCl_2$, 20 mM taurine, 15 mM Na_2-phosphocreatine, 20 mM imidazole, 0.5 mM dithiothreitol, 50 mM MES, 220 mM sucrose, pH 7.4, 380 mOsmol l^{-1}; modified after [69]). Hearts were then separated in fibres and placed in 2 ml ice-cold BIOPS containing 50 μg ml^{-1} saponin and gently shaken on ice for 20 min. Fibres were then washed three times for 10 min in 2 ml ice-cold modified mitochondrial respiration medium MIR05 (0.5 mM EGTA, 3 mM $MgCl_2$, 60 mM K-lactobionate, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, 160 mM sucrose, 1 g l^{-1} bovine albumine serum, pH 7.4, 380 mOsmol l^{-1}) [29, 69].

Directly before experimentation, a subsample of about 10 mg fibres was blotted dry, weighed and introduced into the oxygraph sample chambers.

Mitochondrial respiration

Mitochondrial respiration was recorded using Oroboros Oxygraph-2 k™ respirometers (Oroboros Instruments, Innsbruck, Austria) and measured as weight-specific oxygen flux [pmol O_2 (mg fresh weight sec)$^{-1}$] calculated in real time using Oroboros DatLab Software 5.2.1.51 (Oroboros Instruments, Innsbruck, Austria).

All analyses were performed at the respective incubation temperatures, with cO_2 in a range from ~370 nmol ml^{-1} (100% air saturation) to 100 nmol ml^{-1} and PCO_2 at atmospheric levels.

A substrate-uncoupler-inhibitor titration (SUIT) protocol was used on the permeabilised cardiac fibres to investigate the partial contributions of the single components of the phosphorylation system [69]). NADH - Coenzyme Q oxidoreductase (Complex I, CI) and Succinate dehydrogenase (Complex II, CII) substrates (10 mM glutamate, 2 mM malate, 10 mM pyruvate and 10 mM succinate) were added. Saturating ADP (3 mM) was added to stimulate oxidative phosphorylation (OXPHOS). Cytochrome c (10 μM) was added to test the integrity of the outer membrane. Respiration state IV^+ was measured by addition of atractyloside (0.75 mM) or oligomycin (6 μM) (for Polar cod and NEAC respectively) and step-wise (1 μM each) titration of carbonyl cyanide *p*-(trifluoromethoxy) phenylhydrazone (FCCP) was used to uncouple mitochondria (ETS). Complex I, Complex II and Coenzyme Q – cytochrome c reductase (Complex III, CIII) were inhibited by the addition of rotenone (0.5 μM), malonate (5 mM) and antimycin a (2.5 μM), respectively. Lastly the activity of the Cytochrome c oxidase (Complex IV, CIV) was measured by the addition of the electron donor couple ascorbate (2 mM) and *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPD, 0.5 mM).

All chemicals were obtained from Sigma-Aldrich (Germany).

Data analysis

Mitochondrial respiration rates are expressed per mg fresh weight of cardiac fibres and the values are given as means ± S.E.M. OXPHOS coupling efficiency was calculated as [(OXPHOS-State IV^+) $OXPHOS^{-1}$] after Gnaiger [70].

Normal distribution of the data was assessed by Shapiro-Wilk test and homoscedasticity was evaluated by F-test or Bartlett test in case of two or more groups, respectively. Differences between PCO_2 treatments within the same temperature treatment were evaluated by Student's *t*-test (with Welch's correction in case of non-homoscedastic data). Differences across temperatures in the same PCO_2 treatment were evaluated with one-way ANOVA followed by Tukey's test for the comparison of means.

The level of statistical significance was set at $p < 0.05$ for all the statistical tests.

All statistical tests were performed using R 3.2.0 and the "stats" package [71].

Results

The maximal oxidative phosphorylation capacity (OXPHOS) of permeabilised heart fibres of both species is shown in Fig. 1. In Polar cod, the groups incubated under control PCO_2 showed significantly lower OXPHOS flux in the 0 °C acclimated fish than in all further incubation groups (3 °C, $p = 0.007$; 6 °C, $p = 0.007$; 8 °C, $p = 0.001$). Mitochondrial respiration was at a similar level in the groups incubated at 3, 6 and 8 °C ($p > 0.05$). High PCO_2 levels did not affect OXPHOS, with no differences between the OXPHOS of the groups incubated at the two PCO_2 levels within a temperature treatment ($p > 0.05$). The groups incubated under high PCO_2 displayed fluxes that were similar at 6 and 8 °C ($p > 0.05$) but significantly higher than in the 0 °C incubated group ($p = 0.04$, Fig. 1a).

Temperature had a significant effect on the OXPHOS of NEAC, with fluxes increasing with incubation temperature (control PCO_2: $F = 4.74$, $p = 0.02$; high PCO_2: $F = 3.78$; $p = 0.02$, Fig. 1b). Moreover, the 16 °C/high PCO_2 incubated group showed a lower OXPHOS compared to the 16 °C/control PCO_2 group ($p = 0.03$). This resulted in a more evident plateauing of OXPHOS between 12 and 16 °C in the group incubated under high PCO_2. Comparing the two species, Polar cod had significantly higher OXPHOS capacities than NEAC at both 3 °C ($p = 0.01$, Fig. 1 blue box) and 8 °C (control PCO_2: $p = 0.04$; high PCO_2: $p = 0.04$, Fig. 1 red box).

In both species, state IV^+ was sensitive to temperature (Fig. 2): in Polar cod it remained unchanged in the groups incubated at 0, 3 and 6 °C ($p > 0.05$) but was significantly higher in animals incubated at 8 °C compared to the other incubation groups (6 to 8 °C/control PCO_2: $p = 0.01$; 6 to 8 °C/high PCO_2: $p = 0.04$) as shown in Fig. 2a. Quantifying State IV^+ as a percent fraction of OXPHOS, it was close to 20% and thus lowest in the 3 °C and 6 °C groups of *B. saida*, while at 0 and 8 °C the fraction of State IV^+ exceeded these values about two-fold as shown in Fig. 3.

In NEAC, State IV^+ increased along with incubation temperature (control PCO_2: $F = 5.96$; $p = 0.02$, high PCO_2: $F = 12.43$; $p < 0.001$) as depicted in Fig. 2b, however, State IV^+ increased under high PCO_2 at 8 °C compared to the group incubated under control PCO_2 at the same temperature ($p = 0.02$). Fractional values of State IV^+ in OXPHOS (Fig. 3) for the groups incubated under present levels of CO_2 revealed values close to 20% in the groups incubated to 3 and 8 °C and two-fold higher values after incubation to 12 and 16 °C. In the groups incubated under high PCO_2, State IV^+ of the group incubated at 8 °C showed values similar to the groups incubated to 12 and 16 °C (Fig. 3). In consequence, sensitivity to CO_2 varied with incubation temperature and

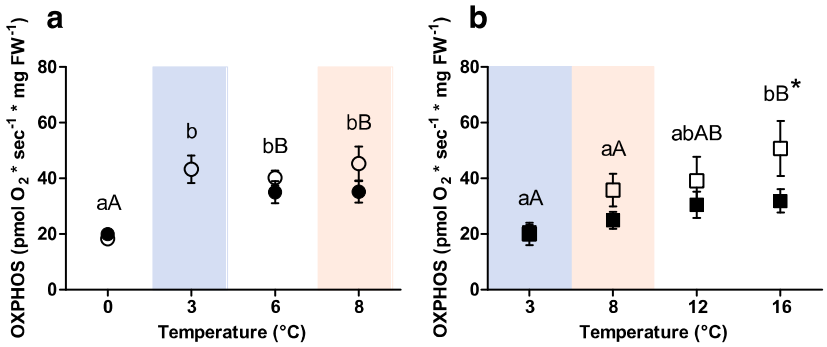

Fig. 1 Maximal oxidative phosphorylation capacity (OXPHOS) of permeabilised heart muscle fibres of (**a**) Polar cod (*B. saida*) and (**b**) NEAC (*G. morhua*). Different letters within panels indicate significant differences ($p < 0.05$) between temperature treatments; *lower case letters*: control PCO_2 (400μatm), *upper case letters*: high PCO_2 (1170μatm), * indicates significant differences ($p < 0.05$) between CO_2 groups at the same temperature. All values are reported as means ± S.E.M. (for *n* refer to Table 1). *Open symbols*: control PCO_2 (400μatm), *filled symbols*: high PCO_2 (1170μatm). *Circles*: Polar cod, *Squares*: NEAC. *Blue box*: cold shared incubation temperature (3 °C), *Red box*: warm shared incubation temperature (8 °C) between the two species

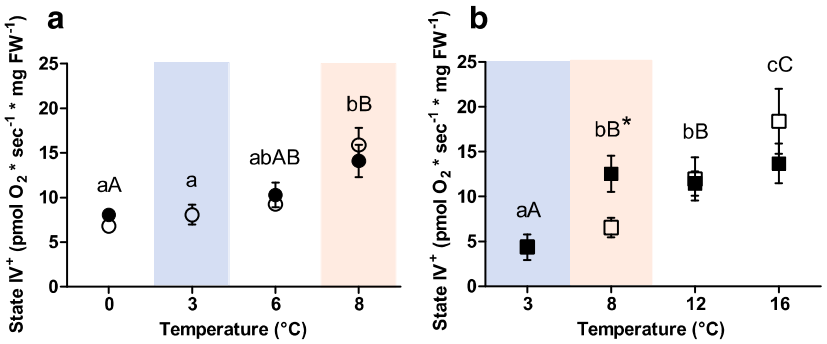

Fig. 2 State IV^{+} of permeabilised heart muscle fibres of (**a**) Polar cod (*B. saida*) and (**b**) NEAC (*G. morhua*). Different letters within panels indicate significant differences (p <0.05) between temperature treatments; *lower case letters*: control PCO_2 (400μatm), *upper case letters*: high PCO_2 (1170μatm), * indicates significant differences (p <0.05) between CO_2 groups at the same temperature. All values are reported as means ± S.E.M. (for n refer to Table 1). *Open symbols*: control PCO_2 (400μatm), *filled symbols*: high PCO_2 (1170μatm). *Circles*: Polar cod, *Squares*: NEAC. *Blue box*: cold shared incubation temperature (3 °C), *Red box*: warm shared incubation temperature (8 °C) between the two species

was maximal but with opposite effects at 8 °C (stimulation of state IV^{+} above controls) and 16 °C (depression of OXPHOS below controls at 16 °C).

OXPHOS coupling efficiency in Polar cod under control PCO_2 was maximal in the group incubated to 3 °C (0.82 ± 0.02), and decreased at 8 °C to values comparable to the 0 °C group (control PCO_2: 0.61 ± 0.03, high PCO_2: 0.58 ± 0.05), mainly because of increased State IV^{+} at 8 °C (Fig. 2, 3 and 4). In NEAC (Fig. 4b), the OXPHOS coupling efficiency was maximal at 8 °C and control PCO_2 (0.81 ± 0.02) and minimal at 16 °C (0.64 ± 0.06). In the groups incubated under high PCO_2, the maximum of OXPHOS coupling efficiency fell to 3 °C (0.77 ± 0.03) and reached its minimum at 8 °C (0.46 ± 0.08) to rise again at 12 °C and 16 °C (0.58 ± 0.05 and 0.56 ± 0.05, respectively). However, these changes in OXPHOS coupling efficiency were not significant (control PCO_2: F = 5.27; p = 0.82, high PCO_2: F = 9.7886, p = 0.072). At 8 °C, the OXPHOS coupling efficiency was significantly lower under high PCO_2 than in the control PCO_2 group (p = 0.003). Comparing the OXPHOS coupling efficiency between the two species, NEAC and Polar cod showed similar values in the 3 °C/control PCO_2 group (Fig. 4 blue box) and at 8 °C/high PCO_2 (p >0.05, Fig. 4 red box), while the coupling efficiency was higher in NEAC incubated at 8 °C/control PCO_2 than in Polar cod incubated under the same conditions (p <0.001, Fig. 4 red box).

The thermal sensitivity of Complex IV also differed between the two species (Fig. 5). In Polar cod, Complex IV capacity rose from 0 to 6 °C (control PCO_2: F = 67.29, p <0.001) and decreased between 6 °C and 8 °C (control PCO_2: p <0.001). This trajectory was only present as a non-significant trend in the groups incubated under high PCO_2 (F = 3.88, p = 0.10) because of the non-significant decrease of the mean

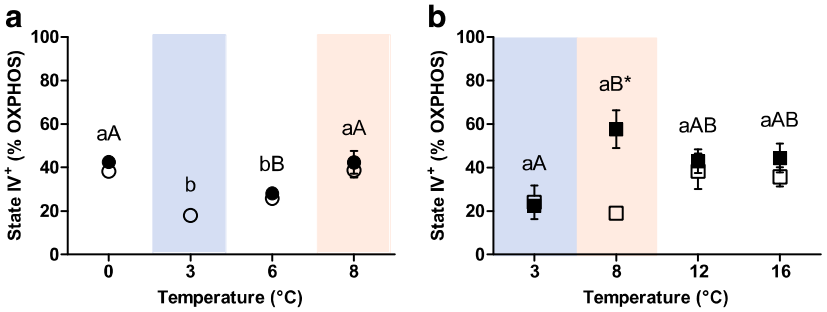

Fig. 3 Percentage of oxygen consumed by State IV^{+} in relation to OXPHOS in permeabilised heart muscle fibres of Polar cod (*B. saida,* panel **a**) and NEAC (*G. morhua,* panel **b**). Different letters within the panels indicate significant differences (p <0.05) between temperature treatments; *lower case letters*: control PCO_2 (400μatm), *upper case letters*: high PCO_2 (1170μatm), * indicates significant differences (p <0.05) between CO_2 groups at the same temperature. All values are reported as means ± S.E.M. (for n refer to Table 1). *Open symbols*: control PCO_2 (400μatm), *filled symbols*: high PCO_2 (1170μatm). *Circles*: Polar cod, *Squares*: NEAC. *Blue box*: cold shared incubation temperature (3 °C), *Red box*: warm shared incubation temperature (8 °C) between the two species

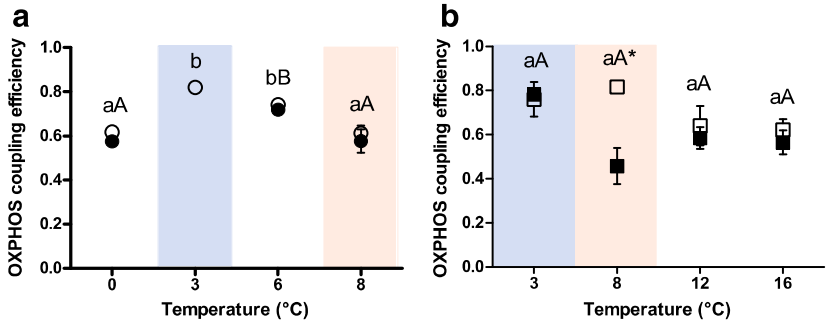

Fig. 4 OXPHOS coupling efficiency in permeabilised heart muscle fibres of (**a**) Polar cod (*B. saida*) and (**b**) NEAC (*G. morhua*). Different letters within panels indicate significant differences (p <0.05) between temperature treatments; *lower case letters*: control PCO_2 (400μatm), *upper case letters*: high PCO_2 (1170μatm), * indicates significant differences (p <0.05) between CO_2 groups at the same temperature. All values are reported as means ± S.E.M. (for n refer to Table 1). *Open symbols*: control PCO_2 (400μatm), *filled symbols*: high PCO_2 (1170μatm). *Circles*: Polar cod, *Squares*: NEAC. *Blue box*: cold shared incubation temperature (3 °C), *Red box*: warm shared incubation temperature (8 °C) between the two species

capacity of Complex IV at 6 °C/high PCO_2 compared to control PCO_2 at the same temperature ($p = 0.09$). In NEAC, Complex IV capacity increased with increasing temperatures in the groups incubated under control PCO_2 ($F = 3.25$, $p = 0.05$), but not in the groups incubated under high PCO_2 ($F = 2.18$, $p = 0.12$). At 16 °C, the capacity of NEAC Complex IV was lower under high PCO_2 (p =0.099) than under control PCO_2. Comparing the two species, the capacity of Complex IV was similar (non-significant differences) in all shared treatments (3 °C/control CO_2, 8 °C/control CO_2 and 8 °C/high CO_2: p >0.05, Fig. 5 blue and red boxes).

Discussion

Our study shows differences in mitochondrial metabolism between a cold-adapted Arctic and a cold-acclimated sub-Arctic fish from the same area, potentially leading to differences in acclimation capacities to ocean acidification and warming.

Mitochondria from permeabilised heart fibres appeared to be affected mainly by the incubation temperature while high levels of CO_2 significantly affected mitochondrial respiration only in NEAC (*Gadus morhua*) and mainly at the highest investigated temperature (16 °C). NEAC OXPHOS and Complex IV capacities decreased under elevated CO_2 at high temperature, although the latter only as non-significant trend. This suggests that the noxious effects of high PCO_2 are stronger at the upper end of the thermal window and might affect the heat tolerance of NEAC [2, 17]. Furthermore, proton leak at 8 °C was higher in the group incubated under high PCO_2 than in the control PCO_2 group, indicating that overall mitochondrial efficiency might be affected through alterations of membrane characteristics. Elevated PCO_2 is reported to inhibit Citrate Synthase and

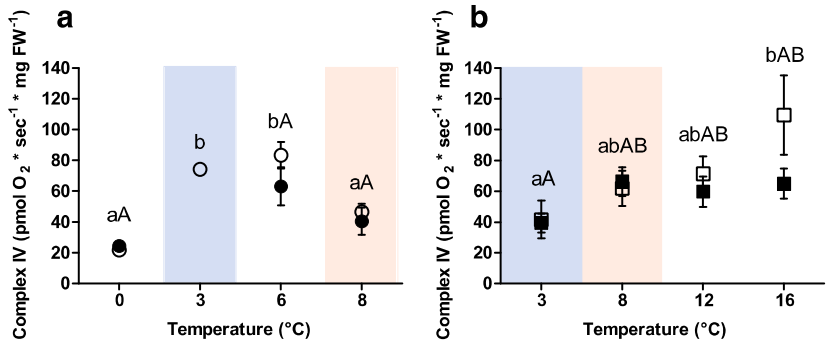

Fig. 5 Complex IV (Cytochrome c Oxidase) capacity. Panel **a**: permeabilised heart muscle fibres of Polar cod (*B. saida*). Panel **b**: permeabilised heart muscle fibres of NEAC (G. *morhua*). Different letters within the panels indicate significant differences (p <0.05) between temperature treatments; *lower case letters*: control PCO_2 (400μatm), *upper case letters*: high PCO_2 (1170μatm). All values are reported as means ± S.E.M. (for n refer to Table 1). *Open symbols*: control PCO_2 (400μatm), *filled symbols*: high PCO_2 (1170μatm). *Circles*: Polar cod, *Squares*: NEAC. *Blue box*: cold shared incubation temperature (3 °C), *Red box*: warm shared incubation temperature (8 °C) between the two species

Complex II in mammals and fish [40, 72, 73] with subsequent stimulation of the mitochondrial anaplerotic pathways to overcome this inhibition [40, 74]. The difference in sensitivity of the two species to elevated levels of CO_2 could be related to differences in preferential metabolic pathways, with Polar cod (*Boreogadus saida*) relying more than NEAC on anaplerotic pathways that feed directly into Complex I such as the oxidation of glutamate, pyruvate or palmitoyl carnitine [40, 73]. Further investigation, especially at the genetic level is needed. Furthermore, it is still unknown whether and to what extent elevated PCO_2 might alter the membrane characteristics and contribute to proton leak.

In Polar cod, OXPHOS of the groups incubated at 3-6-8 °C was higher at the respective incubation temperature than OXPHOS of the 0 °C treatments while the OXPHOS coupling efficiency was highest in the 3 °C group and lowest in the 0 and 8 °C groups. This indicates an optimum temperature for ATP production efficiency between 3 and 6 °C. At lower and higher temperatures, the increased proton leak in relation to OXPHOS created a less favourable ratio between ATP produced and oxygen consumed. These findings match those by Drost et al. [75], where heart rate of acutely warmed Polar cod increased until a first Arrhenius breakpoint at 3 °C. Heart rate still increased further but at a lower rate until 8 °C, passing a second break temperature. In our study, 8 °C corresponds to the highest rate of proton leak, and lowest Complex IV capacity, implying a direct participation of mitochondria in the thermal responses of the heart. The close similarity between the data from the acute study of Drost et al. [75], our 4-months incubation study and a study on behavioural thermal preference from Schurmann and Christiansen [76] indicates preferred temperatures of 3–6 °C within a thermal gradient from 0 to 8 °C for Polar cod, suggesting that Polar cod have only limited abilities to acclimate to higher temperatures.

In contrast, NEAC OXPHOS continued to increase with long-term incubation temperatures to even above those experienced within the natural habitat. This appears to occur without compromising OXPHOS coupling efficiency and reveals a higher acclimation potential than Polar cod, in line with the overall distribution area of Atlantic cod from temperate to (sub-) Arctic waters. This apparent plasticity is in line with the findings by Zittier et al. [77] in which NEAC specimens acclimated to 15 °C displayed critical temperatures (Tc, defined as the onset of the anaerobic metabolism, cf. Frederich & Pörtner [78]) about 10 °C higher than specimens kept at ambient temperature (4 °C). In Polar cod, the high proton leak at 8 °C is the main cause of reduced mitochondrial efficiency (OXPHOS coupling efficiency). This increase in proton leak can be caused by loss of membrane integrity in response to changes in membrane fluidity [7, 79]. In a previous study, Martinez et al. [80] found increased proton permeability of the inner mitochondrial membrane of the Antarctic silverfish *Pleuragramma antarcticum* after warming. In addition, Strobel et al. [40] found that this may be due to an unchanged saturation index of the mitochondrial membrane, observed in liver of the Antarctic *Notothenia rossii* after warm acclimation. These findings suggest a limited ability of Antarctic stenothermal fish to acclimate to temperature changes. Similar patterns may constrain acclimation of cold-adapted Arctic fish. The decreased capacity of Complex IV at 8 °C in Polar cod implies that the interactions between the inner membrane and embedded enzymes may also be affected by high temperatures [80, 81]. In NEAC, proton leak was lower than in Polar cod and reached 40% of OXPHOS at 12 °C, while in Polar cod the same relative values were found at 8 °C under control PCO_2. A strong thermal response of proton leak may reflect high temperature sensitivity of the organism [52, 82–84], and thus a higher baseline proton leak combined with its steeper increase upon warming may point towards a stronger degree of cold adaptation in Polar cod.

The findings in this study contrast earlier results obtained in isolated mitochondrial suspensions where mitochondria remained fully functional beyond whole organism heat limits [82, 85]. The present findings suggest that mitochondria may display wider thermal limits in suspensions than when embedded in permeabilised fibres. Mitochondria in permeabilised fibres may still interact with other cellular organelles and are thus integrated into a more complex system than are isolated mitochondria. These considerations suggest that thermal tolerance is more constrained in permeabilized fibres than in isolated mitochondria. Such findings may thus be in line with the assumed narrowing of thermal windows once molecular and mitochondrial functions are integrated into larger units up to whole organism [86]. While the experiment was carried out at non-limiting PO_2 in the media (>100 nmol ml^{-1}) [87], diffusion gradients of oxygen and/or other substances within the permeabilised cardiac fibres may cause this hierarchy in thermal constraints. In a study on growth, mortality and standard metabolic rates (SMR) of the same Polar cod and NEAC as examined in this study, Kunz et al. [61] found higher SMR in Polar cod than in NEAC at the same incubation temperatures. This is mirrored in the mitochondrial respiration presented in this study, where OXPHOS capacity in Polar cod was larger than in NEAC at both 3 and 8 °C. In Polar cod, the SMR of the 3 and 6 °C groups were lower than in the groups incubated at 8 °C, which is mirrored in the pattern of cardiac State IV^+ respiration. At 8 °C the OXPHOS coupling

efficiency (i.e. ATP production efficiency) decreased as State IV^+ increased and the capacity of Complex IV decreased. Maybe these findings indicate decreased cardiac mitochondrial efficiency that may limit cardiac function and promote heart failure, which is consistent with a drop in cardiac function [75] and the onset of heart failure in Polar cod at 8 °C. At this temperature, oxygen demand and mortality increased, and growth decreased in this species [61]. In fact, the estimated decrease in ATP production efficiency at 8 °C was paralleled by a reduced feed conversion efficiency and concomitant increase in SMR. This likely indicates a shift in energy allocation due to an impaired balance between energy production and demand, e.g. due to increased mitochondrial proton leak (see [88] for review). According to these findings, 8 °C is close to the long-term upper thermal tolerance limit for the Svalbard population of Polar cod, which is again in line with the observed increased mortality [61].

In NEAC, the parallel rise of whole organism SMR and cardiac fibre OXPHOS and the parallel decrease of OXPHOS and SMR at high PCO_2 compared to controls at 16 °C indicates that cardiac mitochondrial function is adjusted to the level of whole animal energy demand at different incubation temperatures and that the effects of high PCO_2 are greatest close to the upper thermal limit. Thermal constraints setting in at whole animal level may again relate to the thermal sensitivity of cardiac mitochondrial function [28, 29, 32, 33]. The fact that first performance limitations are observed in the 16 °C/high PCO_2 incubation may not be of direct relevance for the Svalbard stock of NEAC over the next century, but marks a potential southern distribution limit for the Barents Sea and Norwegian Sea.

Polar cod is a cold adapted species and the constraint on cardiac mitochondrial metabolism at 8 °C, concomitant with increased mortality indicates that the animal's thermal window matches its current habitat temperature range. In contrast, adult NEAC show the ability to broaden their thermal window beyond the present sub-Arctic habitat temperatures (see above). Because of the habitat temperature range of the two species is similarly wide but shifted to lower temperatures in Polar cod, combined with the high metabolic baseline cost (SMR) of Polar cod the two species may be classified as cold-adapted (Polar cod) or cold-acclimated (NEAC) eurytherms. NEAC appear to be much more plastic than Polar cod, thus, Polar cod may be more vulnerable to future ocean conditions than NEAC.

Conclusions

Future ocean acidification and warming may impair cardiac mitochondrial function of Polar cod (*Boreogadus saida*) and Northeast Arctic cod (NEAC, *Gadus morhua*) in somewhat different ways. In Polar cod, high temperature (8 °C) increases proton leak and thereby decreases ATP production efficiency, while high CO_2 levels did not have a significant effect. In NEAC, mitochondrial respiration remained functional at higher temperatures, but capacity was depressed by the combination of high temperature and high PCO_2. Furthermore, in NEAC, incubation temperature leads to variable mitochondrial response patterns under elevated PCO_2. The causes of the different responses to elevated PCO_2 in the heart of these two species remain to be identified, for example, the role of anaplerotic pathways and their regulation should be further investigated.

As a result of the degree of cold adaptation, Polar cod display high metabolic maintenance costs (indicating that it is cold-eurythermal) and low acclimation capacity, while NEAC is cold acclimated and benefits from a lower rate of metabolism and a higher plasticity to acclimate to increasing temperature. As a consequence, mitochondrial function of NEAC hearts may be less constrained by rising temperatures than Polar cod, indicating that NEAC could outperform and possibly replace Polar cod in the waters around Svalbard if ocean warming and acidification further increase towards the conditions predicted for the end of the century (8 °C and 1170μatm PCO_2). Since Polar cod has a key role in Arctic ecosystems [48], temperature driven changes in the distribution of this species can be an important component in the impacts of climate change on Arctic ocean ecosystems.

Acknowledgements
We thank Silvia Hardenberg, Nils Koschnick, Timo Hirse, Isabel Ketelsen and Heidrun Windisch for their support during the incubation and sampling procedures. We acknowledge the project Polarisation (Norwegian Research Council, 214184/F20) for providing Polar cod specimens and the crews of RV Heincke (HE 408) and RV Helmer Hanssen for the animal collection.

Funding
This project was funded by the German Federal Ministry of Education and Research (BMBF, FKZ 03F0655B) within the research program BIOACID phase II and by the PACES program of AWI.

Availability of data and materials
The datasets analysed during the current study and the data regarding the incubation set-up are available from the Open Access library PANGAEA (www.pangaea.de; https://doi.pangaea.de/10.1594/PANGAEA.866369; https://doi.pangaea.de/10.1594/PANGAEA.873536).

Authors' contributions
EL, KK, MS, DS, HOP and FCM designed the study. EL, KK, MS carried out the animal incubations. EL performed the experiment on cardiac mitochondria and all data analyses and interpreted the results together with FCM. EL and FCM drafted the manuscript, KK MS DS and HOP contributed to writing the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval
Experiments were conducted in accordance with the ethical permission number AZ522-27-22/02-00 (113) released by the Senator for Healthcare, Bahnhofsplatz 29, 28195 Bremen on February 21st, 2013 and valid until February 21st, 2017.

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Received: 14 February 2017 Accepted: 30 March 2017
Published online: 14 April 2017

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Acknowledgements - Danksagung

Im Verlauf dieser Doktorarbeit bin ich vielen Menschen begegnet, denen ich auf unterschiedlichste Art und Weise dankbar bin:

Zunächst möchte ich mich gerne bei **Prof. Dr. Hans-Otto Pörtner** dafür bedanken, dass ich meine Doktorarbeit in seiner Sektion durchführen durfte. Vielen Dank für die stete Diskussionsbereitschaft und die vielfältigen Möglichkeiten, die mir während meiner Promotionszeit zuteilwurden.

Prof. Dr. Reinhold Hanel danke ich für die unkomplizierte Zustimmung zur Erstellung eines Gutachtens dieser Arbeit. Darüber hinaus bedanke ich mich bei **Prof. Dr. Wilhelm Hagen** und **Dr. Hauke Flores** für die Bereitschaft mein Promotionskommittee zu ergänzen.

Ein riesengroßer Dank geht an **Dr. Felix Mark** für die exzellente Betreuung, ohne die diese Arbeit nicht möglich gewesen wäre, für unzählige Diskussionen, für die Einführungen in die Methoden, fürs kritische Hinterfragen. Vielen Dank Felix für Deine grenzenlose Geduld, für Mahlzeiten vor dem Kühlraum, aufmunternde Worte und natürlich fürs Adoptieren. Ich habe unglaublich viel von Dir gelernt! Weiterhin möchte ich mich gerne bei **Dr. Rainer Knust** für die Betreuung dieses Projekts und das damit verbundene Vertrauen in mich bedanken.

Many thanks are directed to **Prof. Dr. Guy Claireaux** for introducing me into the art of respiration measurements and swimming physiology. Thank you for your guidance and patience during the long days (and nights) in the cooling room.

A warmhearted thank you goes out to **George Hunt**, who afforded me a giant opportunity by inviting me to the ESSAS conference in Seattle that opened up so many more great possibilities! Further, I would like to thank **Torild Johansen** who made it possible that I could participate in a scientific cruise to the Barents Sea. In diesem Zusammenhang möchte ich mich auch gerne bei **POLMAR** für die finanzielle Unterstützung zahlreicher Reisen bedanken.

Darüber hinaus möchte ich mich gerne bei der Sektion **Integrative Ökophysiologie** für die aufregenden Jahre und die tollen Menschen bedanken. Insbesondere möchte ich **Matthias Schmidt** und **Elettra Leo** nennen, ohne deren Kooperation es nicht möglich gewesen wäre, die Experimente durchzuführen. Vielen Dank auch an **Heidrun Windisch**, **Silvia Hardenberg**, **Sebastian Berger**, **Timo Hirse** und **Fredy Veliz Moraleda** für Eure tatkräftige Unterstützung, für kühle Köpfe in hitzigen Momenten und die gute Laune.

Nicht vergessen (wenn auch klitzeklein wenig vernachlässigt) ist die Sektion **Benthopelagische Prozesse**: Vielen lieben Dank für die gute Zeit im D-Gebäude!

Ein besonderer Dank geht an **Bela Buck** und **Bernadette Pogoda** sowie die **AG Marine Aquakultur**. Vielen Dank für Euer Vertrauen, aber auch für den riesengroßen Sack voll mit Geduld.

Ein riesengroßes Dankeschön an **Hauke Flores** für die immerwährende Motivation und Schokipausen. Tusen takk to **Jarle Mork** for inspiring and motivating conversations, for invaluable advice, for believing in me and just for keeping in touch over so many years.

Lena Jakob, Laura Stapp, Tina Sandersfeld, Katharina Michael, Cornelia Kreiss und **Bastian Maus**, ich hatte riesengroßes Glück, so tolle (Büro-)kollegen wie Euch zu erwischen! Vielen Dank für die lustigen Jahre, für unzähligen Gespräche fischiger und nicht-fischiger Natur und einfach fürs Dasein. Vielen Dank auch an **Maj Wetjen** und **Astrid Böhmer**. Ohne Euch beide wäre ich wohl nie richtig in Bremerhaven angekommen. And thank you **Nina Paul** and **Kseniya Vereshchagina** for making the very, very last period of my thesis much more pleasant.

Danke **Miguel Tripp**, **Dragos Chirila, Verena Merk** und **Bérenger Colsoul** für die tolle Zeit in Bremerhaven. Miguel, unsere verrückte Schweden-Reise mit Bombe an Bord werde ich wohl niemals vergessen. Dragos, vielen Dank für die langjährigen gemeinsamen Joggingtouren, die gerne hochfrequent durch Eis am Deich ersetzt wurden, für die vielen tutorials im positiv denken und natürlich für Deine unbeschreibliche Geduld und Hilfe beim Formatieren. Verena, vielen Dank für sonntägliche Frühstückssessions, für gemeinsames Kopfzerbrechen und eine großartige Zeit.

Lars Beierlein und **Ole Valk** ich weiß garnicht, ob ich soweit gekommen wäre, wenn ich Euch nicht kennengelernt hätte. Vielen Dank dafür, dass Ihr so verrückt seid, wie Ihr seid und dass Ihr mich daran teilhaben lasst.

Mike Goodliff, our common time in Bremerhaven was short, but I can`t think of it to be any better than it was. Thank you for making me laugh even at times when I felt blue, for countless amazing trips including near-death experiences, for teaching me essential English words, for goobering around and for being my favorite English. Gelater!

Katrin Reul, **Marina Courtney**, **Otti** und **Eva Heddergott**, Eure langjährige Freundschaft bedeutet mir unendlich viel. Danke, dass Ihr mir auch durch die Zeit der Doktorarbeit treu geblieben seid und stets mein sicherer Hafen wart. Bald ist die neue, alte Kristina zurück, ich versprech's!

Nicht zuletzt möchte ich mich bei meiner **Familie** bedanken, die mir diesen Weg überhaupt erst ermöglicht hat. Allen voran geht mein tiefer Dank an meinen Vater Karl-Lothar. Danke Papa. Ohne Deine Liebe, Deine stetige Inspiration und die Vermittlung Deiner Neugier wäre ich nicht da, wo ich heute bin. Vielen Dank an meine Mama Petra, meinen Bruder Alexander und Nadja dafür, dass ihr immer da seid!

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