Antarctic Fishes in a Changing Climate:
A Comparative Approach to Predicting Species-Specific Futures

By

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Dedication

For the little girl inside me, and for the little ones inside us all.

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Abstract

The polar regions are experiencing climate change at the fastest rates on Earth and serve as bellwethers for the profound threats facing species, ecosystems, and physical processes worldwide due to uncurbed anthropogenic greenhouse gas emissions. My dissertation research focuses on early life stages of Antarctic fishes, which are thought to be particularly vulnerable to climate change due to their unique evolutionary history and specialization to their stenothermal habitat. I used a comparative framework, examining four closely related species in the Nototheniidae family – *Trematomus bernacchii*, *Trematomus pennellii*, *Trematomus nicolai*, and *Pagothenia borchgrevinki* – to understand how subtle interspecific variation in traits may impact species-specific performance under projected future ocean conditions.

I first measured basal characteristics across the four species, as very little is known about Antarctic fishes at young life stages, focusing on metabolic traits and the exploration-avoidance axis of behavior, two key dimensions of species fitness and drivers of niche differentiation. While basal metabolic demands appeared relatively conserved across species at the juvenile life stage, I found divergent behavioral strategies that could be a critical driver of niche differentiation in Antarctic fish assemblages. *T. bernacchii* and *T. pennellii* showed risk-prone behavior, *T. nicolai* showed avoidant behavior, and *P. borchgrevinki* showed cautious exploratory behavior. I also observed a potentially conserved freezing strategy in response to novelty, which, when paired with *in situ* observations, indicates that freezing may be an important predator avoidance strategy in these fishes.

I then focused on the two 'risky' species – *Trematomus bernacchii* and *Trematomus pennellii* – to explore how acclimation to projected future ocean warming and ocean acidification conditions may impact their risk-prone behavior. While acclimation to warming and elevated pCO_2 affected behavior in both species, the effect sizes of pCO_2 were small, and warming was the driving force behind behavioral modifications. In both species, fishes acclimated to ocean warming conditions demonstrated reduced exploratory activity and showed indications of neophilia. These responses amplified over time, and *T. pennellii* demonstrated a stronger response (i.e., effect sizes) in both behaviors. Consistent with previous physiological and behavioral studies, while limited, our results support the inference that *T. pennellii* have a particularly risk-prone strategy when faced with novelty that is amplified when acclimated to warming.

My final chapter proposes a novel 'ice reef' framework and emphasizes how three-dimensional ice habitat formed by platelet, anchor, and brinicle ice may function as critical nursery and refugia habitats for young polar fishes. Drawing on *in situ* observations and the literature, I discuss the recurring behavioral, physiological, and morphological features across a diversity of polar fishes, suggesting ice-associated and ice-obligate life history strategies may be much more widespread than previously acknowledged. As climate change rapidly alters ice phenology and stability, the loss of ice reefs could jeopardize fish recruitment, community resilience, and key ecosystem services. This perspective underscores the urgent need to study ice reefs before they disappear altogether.

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CHAPTER 1

General Introduction

An Abbreviated History of Climate Change Disinformation

The greenhouse effect – and its potential implications – was first described by an American suffragist and amateur scientist, Eunice Newton Foote, in 1856 (Jackson, 2019). By filling glass cylinders with different gases and exposing them to sunlight, she empirically demonstrated that carbon dioxide (CO_2) absorbed and retained heat significantly better than air or other gases, especially when combined with water vapor (Foote, 1856). Noting that "an atmosphere of that gas would give to our Earth a high temperature," Foote was the first to directly connect the relationship of atmospheric CO_2 and Earth's climate. It was not until five years later that John Tyndall – an Irish physicist who has long been credited with the discovery of the greenhouse gas effect – made a similar suggestion (Tyndall, 1861). As a professionally trained scientist with access to the newest technologies, Tyndall was able to differentiate the effects of infrared light on the heat absorption and retention of gases (Jackson, 2019). Decades later, the Swedish scientist Svante Arrhenius was the first to mathematically model the greenhouse effect; he estimated that doubling atmospheric CO_2 would lead to a global temperature rise of about 5-6°C (Arrhenius, 1896).

Despite this early scientific evidence, it was not until the mid-20th century – when fossil fuel corporations themselves began investigating the effects of greenhouse gas emissions – that the catastrophic implications of greenhouse gas emissions were fully understood (Oreskes and Conway, 2010; Westervelt, 2016). Through the 1950's and

1960's, academic, government, and industry scientists concurrently and independently built a firm understanding of the risks of climate change. Scientists sounded the alarm bells, but they were stifled. For example in 1989, an analyst employed by Shell warned that, "global warming could challenge the very fabric of the world's ecological and economic systems" (International Energy Agency, 1989). In fact abundant internal documents from within ExxonMobil, Shell, British Petroleum, Chevron, and ConocoPhillips dating back to the early 1970's reveal explicit recognition of greenhouse gas emissions effects on global temperatures, sea-level rise, and ecosystem stability (U.S. Senate & House Committees, 2024). Even before mainstream scientific consensus coalesced, internal scientists at ExxonMobil and Shell developed advanced climate models that, decades later, proved to accurately predict global warming trends (Supran *et al*., 2023; Supran and Oreskes, 2017). In blatant disregard, company executives chose to fund aggressive disinformation campaigns designed to manufacture uncertainty around climate science and global warming (Mann, 2012; Oreskes and Conway, 2010; U.S. Senate & House Committees, 2024; Westervelt, 2016). With billions of dollars of lobbying, coordinated political pressure, crafted media narratives, and intentional obfuscation and disinformation, oil and gas corporations successfully manufactured doubt that delayed climate action by decades (Brulle, 2018; Doreian and Mrvar, 2022; Oreskes and Conway, 2010; Supran and Oreskes, 2017; Westervelt, 2016). To this day, even as scientist warnings have escalated into desperate pleas, fossil fuel companies continue to fund anti-regulation lobbying, push to open protected regions to extraction, and heavily invest in fossil fuels (U.S. Senate & House Committees, 2024).

An extraordinary amount of evidence documents that a relatively small group of fossil fuel industry executives, lobbyists, and PR strategists – fewer than a hundred – successfully gaslit the world for decades (Supran *et al*., 2023; Oreskes and Conway, 2010; U.S. Senate & House Committees, 2024; Verlie, 2024; Westervelt, 2016). Their actions constitute a profound abuse of power, akin to abuse on the global scale, where deliberate deception and manipulation has deeply harmed hundreds of millions of people on Earth via climate distress (anxiety, grief, anger), PTSD, loss of housing and livelihoods, bodily injury, and death, particularly within marginalized communities (Clayton, 2020; Clayton *et al.*, 2017; Doherty, 2018; Hickman *et al*., 2021; Manning and Clayton, 2018). The World Health Organization projects a quarter million additional deaths annually between 2030 and 2050 due to climate-induced malnutrition, illness, and heat stress (WHO, 2014). This is climate violence (Verlie, 2024). Not only is climate violence inherently rooted in colonial, patriarchal, racist, capitalist, and ableist systems of oppression (see: affective climate violence, petro-masculinity, greenhouse gaslighting) – it also synergizes with those oppressions, further amplifying climate vulnerability and harm (Daggett, 2018; Doherty, 2018; Heglar & Westervelt, 2020; Manning and Clayton, 2018; Pellow, 2018; Sadler, 2023; Westervelt, 2016). Intersectional climate justice movements highlight the critical need for true climate solutions that directly address these interconnected injustices (Heglar & Westervelt, 2020; Miranda *et al*., 2011; Sadler, 2023; Washington, 2020).

Despite clear scientific consensus and urgent calls for action, decades of deliberately manufactured doubt and climate gaslighting have severely hindered global efforts to mitigate emissions. We have thus far failed to curb anthropogenic emissions

sufficiently to stay below the 1.5°C - 2°C warming benchmarks set by the Paris Climate Accord (IPCC, 2021; UNFCCC, 2015); global greenhouse gas emissions continue to rise.

Global Consequences of Climate Change in Polar Regions

Oceans have absorbed 90% of the excess heat associated with anthropogenic greenhouse gas emissions in recent decades (IPCC, 2021). Polar oceans account for disproportionately high rates of heat and CO_2 uptake – for example, at only 25% of global ocean surface area, the Southern Ocean accounted for 40-60% of global ocean heat gain between 2005 and 2017 (Gruber *et al*., 2019; IPCC, 2019). Under the Intergovernmental Panel on Climate Change (IPCC) high-emission SSP5-8.5 scenario (i.e., the business-as-usual scenario), global mean sea surface temperatures (SST) are projected to rise by approximately 2.9°C (with a likely range of 2.0°C to 4.1°C) by the end of the 21st century (1995–2014 to 2081–2100; IPCC CMIP6 AR6). Global surface air temperatures are projected to rise by 4.4°C (with a likely range of 3.3°C to 5.7°C) by the end of the 21st century compared to the pre-industrial baseline (1850–1900 to 2081–2100; IPCC CMIP6 AR6 SSP5-8.5). Arctic air temperatures are expected to warm by 5.4°C (with a likely range of 2.8°C to 10.0°C), much higher than the global mean, while Antarctic air temperatures are expected to warm by 2.7°C (with a likely range of 1.8°C to 3.4°C), on par with the global mean (1995–2014 to 2081–2100; IPCC CMIP6 AR6 SSP5-8.5). The comparatively lower projected warming in the Antarctic is thought to be due to strong oceanic heat uptake and the buffering effect of the Southern Ocean surrounding Antarctica, although it is critical to note that predictions on Antarctic regions suffer from a dearth of available data and more recent studies document rapid warming

in the Antarctic above modeled projections (Casado *et al*., 2023; Clem *et al.*, 2020; Gorodetskaya *et al*., 2023; Meredith and King, 2005; IPCC, 2021).

Both poles are warming much more rapidly than the global average. Between 1979 and 2021, the Arctic warmed at a rate nearly four times that of the global average (0.73°C compared to 0.19°C per decade) (Rantanen *et al*., 2022). Based on isotopic analyses of ice cores, Antarctica is estimated to be warming at a rate between 0.22°C and 0.32°C per decade, about 50% faster than earlier climate models projected (Casado *et al*., 2023). Critically, warming is not uniform across the continent of Antarctica and the West Antarctic Peninsula (WAP) and the West Antarctic Ice Sheet (WAIS) are of primary concern. Between 1950 and 2000, the WAP warmed by 2.5°C, approximately 0.50°C per decade (Turner *et al*., 2005). Similarly, between 1958 and 2010, average temperatures at Byrd Station on WAIS warmed by 2.4°C, approximately 0.46°C per decade (Bromwich *et al*., 2013). East Antarctica – including the vast interior of the continent – has historically shown more stable temperatures, recent studies are showing alarming rates of warming. Between 1989 and 2018, the South Pole warmed 0.61± 0.34°C per decade, more than 3x the global average (Clem *et al*., 2020).

Warming in the poles is triggering several positive feedback loops that amplify further change. In the Arctic, these processes are well documented and widely acknowledged. Most notably is the albedo effect, which was first quantified by Soviet climatologist Mikhail Budyko and American meteorologist William D. Sellers (Budyko, 1969; Sellers, 1969). This positive feedback loop is driven by the albedo – the fraction of sunlight that is reflected vs. absorbed – of different surfaces. As sea ice disappears, it exposes dark open water, reducing the albedo from ~0.85 to <0.1, in turn absorbing

significantly more solar radiation, which then further warms the ocean and melts more ice (Elias, 2021; Kashiwase *et al.*, 2017; Winton, 2008). A similar feedback loop is seen in permafrost – ground that is frozen over many years – in Arctic terrestrial systems. As rising Arctic air temperatures thaw permafrost, it releases ancient CO_2 and methane that has been 'stored' in the frozen ground for thousands to tens of thousands of years (Schuur *et al*., 2015). This input of greenhouse gases further amplifies the greenhouse gas effect, which then forces more permafrost thawing, and so on (Elias, 2021; Schuur *et al*., 2015). The albedo effect and permafrost thaw feedback loops drive Arctic amplification – the process that describes the significantly faster rates of warming in the Arctic compared to lower latitudes (Elias, 2021; IPCC, 2021; Post *et al*., 2019).

While it has long appeared that the Antarctic is not experiencing the same amplification as the Arctic, recent studies report that similar positive feedback loops are indeed taking hold in the Antarctic, amplifying warming and melting. After decades of relative stability (compared to the Arctic), Antarctic sea ice extent has plummeted since 2016, with multiple record-low ice cover seasons in recent years (Purich and Doddridge, 2023). This alarming trend indicates that the Antarctic has entered a new sea ice state, potentially due to altered processes that underlie Antarctic sea ice cover (Purich and Doddridge, 2023). In addition to these concerning new sea-ice dynamics, oceanic positive feedback loops are also driving ice shelf melting and destabilization in the Antarctic. Ice shelves – thick, extensive floating platforms of land-based glaciers flowing into the ocean – surround about two thirds of the Antarctic coastline (Fürst *et al.*, 2016). While ice shelves are already 'destined' to melt as they are already flowing into the sea, recent studies document that shockingly warm bottom waters – up to 2°C, nearly 4°C

warmer than the freezing point of seawater – are intruding onto continental shelves and rapidly melting ice shelves from the underside (Jenkins *et al.*, 2010; Lauber *et al*., 2023; Pritchard *et al*., 2012; Schmidt *et al.*, 2023). Importantly, the reduction of sea-ice cover is a key mechanism facilitating the upwelling of warm bottom waters onto the continental shelves, along with strengthening westerlies (Lauber *et al*., 2023; Pritchard *et al*., 2012). The implications of amplified melting and destabilization of Antarctic ice shelves cannot be understated, because ice shelves play a critical role in stabilizing the entire Antarctic Ice Sheet (AIS) by buttressing the flow of grounded ice into the sea (Fürst *et al.*, 2016; Fretwell *et al*., 2013; Walker *et al*., 2024). The thinning of ice shelves forces the grounding-line – where the ice shelf meets the seafloor – to retreat, amplifying glacial destabilization and calving; this is known as the Marine Ice Sheet Instability (MISI) hypothesis and was first described in the 1970's (Mercer, 1978).

For many decades, most attention has been focused on the West Antarctic Ice Sheet (WAIS). For example, extensive international collaborative efforts have focused on the Thwaites Glacier, located on the WAIS, making it the most well-studied glacier in Antarctica (ITGC, 2018). Thwaites, also dubbed the "Doomsday Glacier" in popular media, currently accounts for 4% of global sea level rise, and flow has nearly doubled since the 1990's (NSIDC, 2018). Thwaites is of such concern because of its role as the "weak underbelly" of WAIS; it is grounded on a seafloor ride – a pinning point – inland of which the bedrock slopes downward in a retrograde slope. This specific seafloor geology is inherently unstable, because as the glacier retreats past the pinning point, Thwaites could rapidly collapse and increase global sea level by ~65 cm. This in turn would also amplify WAIS collapse, which could increase global sea level by 3 to 3.5 m

(Fricker *et al*., 2025; Klose *et al*., 2024; IPCC, 2021; NSIDC, 2018).

Until nearly 2020, it was thought that the East Antarctic Ice Sheet (EAIS) was relatively stable compared to the WAIS, from cold shelf waters limiting melting, recent studies have documented the vulnerability of the EAIS to similar oceanic forcing as the WAIS (Clem *et al.*, 2020; Jordan *et al.*, 2023; Rignot *et al*., 2019; Shen *et al*., 2018; Stokes *et al*., 2025). At the Fimbul Ice Shelf, located on the coast of the Dronning Maud Land region of Antarctica, warm deep water >0°C has doubled basal melting rates (Lauber *et al*., 2023). In 2022, the Conger-Glenzer glacier, located on the coast of the Mawson Sea on the EAIS, was the first major ice shelf in the EAIS to completely collapse, shocking scientists and ringing alarm bells (Walker *et al*., 2024). Given that the AIS holds 60% of global freshwater – enough ice to raise global sea levels by ~58 m (Morlighem *et al.,* 2020), this unexpected instability of the EAIS is existentially concerning, and is expected to accelerate Antarctica's contribution to global sea level rise. The AIS is already the dominant contributor to sea level rise, but there is significant uncertainty in sea level rise models due to extremely limited data from the Antarctic (IPCC, 2021; Pritchard *et al*., 2012). Our current best estimate under current emissions scenarios project the AIS alone contributing ~0.3 m of sea level rise by 2100 (Fürst *et a*l., 2016; IPCC, 2021).

Critically, the global impacts of climate change at the poles are not limited to sea level rise. Polar oceans act as Earth's global climate regulators, and significantly influence global ocean circulation (IPCC, 2021). A key component of this system is the Atlantic Meridional Overturning Circulation (AMOC), often referred to as the global ocean conveyor belt, a global oceanic current system that transports heat, nutrients,

and CO_2 around the globe. When functioning normally, surface cooling and sea ice formation (via brine rejection) drive overturning circulation in the Arctic and Southern Oceans, forming cold, deep-water currents that regulate Earth's climate (Rahmstorf *et al*., 2015; IPCC, 2021). Unsurprisingly, climate change is interfering with this process; freshwater input from melting polar ice sheets and glaciers attenuate density-driven overturning and has potentially already weakened the AMOC by ~15% since the mid-20th century – an unprecedented slowdown that raises urgent concerns about the stability of global climate regulation (Caesar *et al*., 2021; Caesar *et al*., 2022; Kilbourne *et al.,* 2022). The AMOC is at risk of full collapse; this would cause severe cooling in northern Europe and North America, increased sea-level rise along the North American eastern seaboard, and disrupt precipitation patterns globally (Lenton *et al*., 2019).

Polar warming is also disrupting atmospheric circulation. In the Arctic, the polar jet stream – a fast-moving 'river' of air that forms from the temperature differential between polar and mid-latitude regions – normally helps regulate global weather and moderates extreme weather events by acting as a stable boundary between cold polar air and warmer mid-latitude air masses. Arctic amplification is attenuating the temperature differential between polar and mid-latitude regions, in turn weakening the polar jet stream and increasing meandering and stalling patterns (Francis and Vavrus, 2015). This destabilization is already driving prolonged, extreme weather events across North America, Europe, and parts of Asia, including severe droughts, heatwaves, wildfires, and flooding (Coumou *et al*., 2015; Coumou *et al*., 2018; Francis and Vavrus, 2012; Mann *et al*., 2017; Overland *et al*., 2021). As polar regions continue warming rapidly, these global impacts will only intensify, underscoring the urgent need for

immediate and comprehensive climate action.

Climate change is fundamentally disrupting ecosystems worldwide, and triggering widespread population declines and species extinctions (for reviews, see Pecl *et al*., 2017; Pugnaire *et al.*, 2019; Scheffers *et al.*, 2016; Tylianakis *et al*., 2008). One million species are at risk of extinction – many within mere decades – due to climate change and other anthropogenic activities (IPBES, 2019). Extinction rates – which are continuing to accelerate – are currently at least two orders of magnitude higher than the average extinction rate over the past 10 million years (IPBES, 2019). Species ranges are shifting, generally more poleward, and phenological mismatches are becoming more frequent and severe (Kubelka *et al*., 2022; Kuletz *et al*., 2024; Iler *et al*., 2021; Inouye, 2022; Parmesan and Yohe, 2003; Thackeray *et al*., 2016). These dynamics are altering species interactions and community composition, and ecosystem resilience is suffering (Kubelka *et al*., 2022; Iler *et al*., 2021; Inouye, 2022; Thackeray *et al*., 2016). Polar species and ecosystems are thought to be especially vulnerable, due to their specialization to life in the coldest habitats on Earth (Clarke and Harris, 2003; IPCC, 2023; Laidre *et al*., 2008; Moore and Huntington, 2008; Pörtner *et al*., 2007; Post *et al*., 2019; Rintoul *et al.*, 2018). Antarctic ecosystems support exceptional biodiversity and play pivotal roles in global climate regulation via carbon sequestration and deep ocean circulation, among other processes (Clarke and Harris, 2003; IPPC, 2021; Rintoul *et al.*, 2018).

The Unique Evolutionary History of Antarctic Ecosystems

Antarctic ecosystems are characterized by extremity. Thermally, the environment has historically been among the most stable on Earth, as water temperatures once remained below-freezing year round, ranging from -1.9°C throughout most of the year to -0.5°C in the peak of the austral summer on high-latitude, near-shore shelves (Cheng and Detrich, 2007; Colombo *et al.*, 2015; Cziko *et al.*, 2020; Eastman, 2005). Apart from the stenothermal temperatures, though, most features of Antarctic ecosystems are highly seasonal; photoperiod drastically shifts seasonally from complete darkness May through July to complete sunlight November through February. Sea ice cover seasonally breaks out (Crocker and Wadhams, 1989) and primary productivity follows both the seasonal light cycles and sea ice cover (Loeb *et al*., 1993; Smith *et al*., 2000). Extreme seasonality is thought to drive pulsed productivity cycles, where food is limited for most of the year – especially in winter – but booms in the height of the austral summer (Arrigo *et al*., 1997; Arrigo *et al*., 2010; Clarke, 1988; Clarke *et al.*, 2007; Fach *et al*., 2002; Gutt *et al*., 2021; Iken *et al*., 1997). The phenology of pulsed productivity cycles with sea ice cover is an important area of growing research, as evidence suggests that nutrient and ecosystem dynamics are tightly coupled with sea ice (Brandon *et al*., 2010; Clarke, 1988).

The Antarctic marine ecosystem has a unique evolutionary history. Intensification of the Antarctic Circumpolar Current (ACC) *ca.* 10 – 14 mya created a thermal barrier, through the cooling and glaciation of Antarctica, as well as a physical barrier, through the density-based isolation of water masses and a strong clockwise current (Cheng and Detrich, 2007; Clarke and Crame, 1992; Kennett, 1977). The ACC thus functionally

isolated the Southern Ocean fishes and invertebrates from the rest of the world. Additionally, with glaciation came glacier scouring of the benthos and destruction of inshore habitats; habitat destruction combined with relatively rapid cooling led to the extinction of many Eocene fauna (Eastman and McCune, 2000). The few species that survived diversified to occupy the newly formed niches, leading to multiple of the few known examples of marine species flocks (Bowen *et al*., 2020; Eastman and McCune, 2000).

The environmental extremes of the Southern Ocean – once thought to limit life – have acted as ecological pressures that have strongly shaped species adaptation and persistence in this region (for reviews, see Cheng and Detrich, 2007; Giordano *et al*., 2012; Gutt *et al.*, 2021; Matheson and McGaughran, 2023; Peck, 2018; Pörtner, 2006; Pörtner *et al*., 2007; Verde *et al.*, 2011). Of particular interest in the Antarctic research community has been the question of how ectotherms, which do not actively regulate body temperature, manage to survive in seawater that is significantly colder than the optimal temperature range for biological functions such as enzyme activity, metabolism, and growth, and even below the freezing point of their bodies (Cheng and Detrich, 2007; Peck, 2018; Pörtner, 2006; Verde *et al*., 2011). The vast majority of the species that call the Antarctic home are indeed thermoconformers, with mammals and birds as the exception (Peck, 2018). Numerous adaptations that are thought to enable body function at below-freezing have been described across ectotherms, but primarily in fishes. These adaptations include antifreeze glycoproteins (AFGPs), altered enzyme kinetics, mitochondrial proliferation, more fluid membranes, and reduction of blood

viscosity, among others (Cheng and Detrich, 2007; DeVries and Wohlschlag, 1969; di Prisco *et al.*, 2002; Guderley, 2004; Somero, 2004; Verde *et al*., 2011).

Cold waters hold more gasses, and some adaptations found in Antarctic organisms are thought to have either neutrally or adaptively evolved in response to highly oxygenated waters (Verde *et al*., 2011). Such adaptations include the loss of some inducible heat shock proteins across Notothenioids, the loss of hemoglobin and myoglobin in icefishes (*Channichthyidae*), relatively high concentrations of modified haemocyanin in Antarctic octopuses, and gas exchange across the cuticle of Antarctic sea spiders and the skin of ribbon worms (Lane *et al*., 2018; Oellermann *et al*., 2015; Verde *et al*., 2011). Many species also exhibit slow paces of life, with delayed and extended life history strategies (Agüera *et al.*, 2015; La Mesa and Vacchi, 2001; Sol *et al.*, 2025; Peck, 2002). The same adaptations that are thought to enable Antarctic organisms to survive their extreme habitat (or those that are neutral at below-freezing temperatures) are expected to make them more vulnerable to climate change, because it is thought that these adaptations may malfunction and become maladaptive as temperatures rise above freezing (Montgomery and Clements, 2000; Todgham and Mandic, 2020).

Antarctic Fishes

Of the modern fish fauna in the Southern Ocean, 88% are endemic (Andriashev, 1987). On the high-latitude Antarctic shelves, species belonging to the suborder Notothenioidei are by far the most common, representing up to 77% of the species and 90-95% of fish biomass (Eastman, 2005). There is a wide diversity of notothenioids, with 140 currently described species (Eastman and Eakin, 2021). This dissertation focuses on four of the

most abundant notothenioids in the near-shore waters of McMurdo Sound in the Ross Sea, *Trematomus bernacchii*, *Trematomus pennellii*, *Trematomus nicolai*, and *Pagothenia borchgrevinki*. They are also among the most well-studied adult fish species in the Ross Sea, due to their high abundance and near-shore habitat use.

There remain wide gaps in our understanding of Antarctic fish physiology, ecology, and behavior and what we do know is almost exclusively based on adult life stages. Of the four fishes represented in this dissertation, two are described as benthic (*T. bernacchii* and *T. pennellii*), one epibenthic (*T. nicolai*), and one cryopelagic (*P. borchgrevinki*) at adult life stages. Cryopelagic fish are those that are associated with surface-ice cover. While *T. bernacchii* and *T. pennellii* have a varied diet, there is some evidence of food resource partitioning, which is thought to help reduce interspecific competition in a food-limited ecosystem (Barrera-Oro, 2002). In contrast to their benthic counterparts, *P. borchgrevinki* have a narrow diet of exclusively nektonic (i.e. actively swimming) organisms, especially copepods and amphipods (Eastman and DeVries, 1985). Notably, *P. borchgrevinki* is the only notothenioid currently described as exclusively cryopelagic throughout the life cycle. Previous research on juvenile life stages is limited to less than a handful of studies (Davis *et al.*, 2015; Davis *et al.*, 2018; Johnston *et al*. 2002; Moteki *et al*., 2017; Naslund *et al*., 2021; Naslund *et al*., in review; Voskoboinikova *et al*., 2017) and very little is known regarding juvenile Notothenioid physiology, ecology, and behavior.

The sparse research on young life stages of Antarctic fishes is likely due to limited access – adult notothenioids can be seined, fished, or trapped from above the ice, while young life stages must be collected by SCUBA divers (to ensure the animals

survive collection) or seined from research vessels. Field research on the sea ice in McMurdo Sound is also effectively limited to September through December, when the Antarctic winter storms have subsided, but the sea ice is still thick enough for safe travel. Thus, if species spawn or have ontogenetic habitat shifts that are seasonally modulated at times outside of our field season, they are missed from observations and sampling.

Exclusive study of adult life stages, though, results in major knowledge gaps. From an ecological perspective, when we only study adults we miss important features of life history, such as reproductive strategies (i.e. where, when, and how eggs are laid), and population dynamics (i.e. recruitment and population stability) (Chan, 2018). From the physiological perspective, young life stages are thought to be more sensitive to stress (Pörtner and Peck, 2010; Pankhurst and Munday, 2011), making them the most informative for exposure experiments in terms of understanding vulnerability to ocean change. Finally, from a behavioral perspective, young life stages are known to show distinct behaviors from their adult counterparts (e.g. habitat use, feeding, and escape behaviors) (Gibb *et al.*, 2006; Mittelbach *et al*., 2014). Importantly, studying juvenile life stages helps characterize potential sensitivities that could affect the entire population.

Overarching Aims

The overarching aim of this collection of work is to better describe how juvenile Notothenioid fishes may respond to climate change using behavioral, ecological, and physiological approaches. I focused on understanding niche differentiation among species, as well as potential behavioral strategies that may support species coexistence and metabolic strategies that may inform species capacity to cope with stressors such

as climate change. Together, the goal is to provide a more accurate prediction of species-specific vulnerability to climate change stressors characteristic of predicted future oceans.

The environmental extremity of the Antarctic was once assumed to preclude life, and late 19th century and early 20th century scientists often described the Southern Ocean as a ‘sterile’, ‘barren’, and ‘biologically poor’ ‘marine desert’ (Dodds and Collis, 2017; Howkins, 2010; Lockard, 2015; Rush, 2023). Although we now know that the Antarctic is home to thriving ecosystems with remarkable diversity, modern scientists still often describe and experience the Antarctic as a hostile, inhospitable continent (Blair, 1991; Lockard, 2015). This anthropocentric perspective does a disservice to research, as it limits the questions we ask and how we ask them. When approaching this dissertation, I encourage the reader to intentionally step away from this limiting perspective and to instead approach this body of work from a perspective of abundance – hidden beneath the sea ice a vibrant ecosystem thrives.

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CHAPTER 2

Divergent exploratory-avoidant behavioral strategies may drive niche differentiation in juvenile Antarctic fishes

Abstract

We used an ecological niche theory framework integrating physiological and behavioral ecology to understand interspecific differentiation of juveniles of four Antarctic fishes: *Trematomus bernacchii*, *Trematomus pennellii*, *Trematomus nicolai*, and *Pagothenia borchgrevinki.* We focused on basal metabolic traits and the exploration-avoidance axis of behavior, two key dimensions of species fitness and drivers of niche differentiation. Metabolic metrics included routine metabolic rate (RMR) to understand resting energy requirements and Fulton's condition factor (CF) as a proxy for energy stores; together, these metrics give a baseline understanding of the energetic status of a species. Behavioral assays included the Novel Object Test (NOT) and the Novel Tank Test (NTT) to assess exploratory activity and anxiety. We found relatively low interspecific differentiation in basal metabolic demands – RMR did not differ across species and only *T. nicolai* exhibited higher CF than the other species. This suggests that *T. nicolai* may have greater on-board energy stores available to cope with environmental stressors, although more research is needed to confirm this. While we generally saw behavioral differentiation across species, we did see evidence of a possible conserved freezing strategy in response to novelty, where *T. bernacchii*, *T. pennellii*, and *P. borchgrevinki* reduced their activity after being exposed to a novel object. In both the NOT and NTT, species showed distinct exploratory tendencies, with *T. bernacchii* and *T. pennellii* spending more time in risk-associated zones, *T. nicolai* being more avoidant, and *P.*

borchgrevinki exhibiting cautious exploration. We also observed substantial intraspecific variability in physiological and behavioral traits, particularly in *T. bernacchii*, *T. nicolai*, and *P. borchgrevinki*, suggesting the presence of divergent behavioral phenotypes within species. Examining behavioral syndromes in these fishes is an important next step as behavioral syndromes are thought to be linked to metabolic status, individual fitness, and species and community resilience. Collectively, our findings suggest that while basal metabolic demands appear relatively conserved across species at the juvenile life stage, divergent behavioral strategies could be a critical driver of niche differentiation in Antarctic fish assemblages and in species-specific performance under environmental threats such as climate change.

Introduction

Antarctic fishes

Fishes play a critical role in Antarctic ecosystems. By serving as prey for larger vertebrates, such as other fishes, penguins, and seals, and by acting as the primary predators of the benthos, fishes transfer energy from primary producers to top-level predators (Hureau, 1994). In inshore waters, demersal fishes (those that live near the seafloor) are the most important linkage between primary producers and top-level predators – even more important than krill (Barrera-Oro, 2002). Antarctic fishes show great ecological and dietary diversification and fishes occupy nearly all trophic levels in the ecosystem (La Mesa *et al*., 2004). On the high-latitude Antarctic shelves, species belonging to the suborder Notothenioidei are the most common, representing 77% of the species and up to 90-95% of fish biomass (Eastman, 2005). The majority of notothenioids (130 out of 140 species) are endemic and highly specialized to life in the Antarctic (Eastman and Eakin, 2021).

Fishes have long been a focus of study in the Antarctic research community and, while our understanding of these fishes is continuing to grow, there remain considerable gaps in our understanding of Antarctic fish physiology, ecology, and behavior. Critically, previous physiological and behavioral research on juvenile life stages is limited to a handful of studies (e.g., Davis *et al.*, 2016; Davis *et al*., 2018; Johnston *et al*. 2002; Moteki *et al*., 2017; Voskoboinikova *et al*., 2017) and we have little data on how early life stages differ from their adult counterparts, except see Mandic *et al*. (2022). The sparse research on early life stages of Antarctic fishes is primarily due to limited access – adult notothenioids can be seined, fished, or trapped from above the ice, while embryos,

larvae and juveniles must be collected by SCUBA divers (to ensure the animals survive collection) or seined from research vessels, with mixed success on fish condition and survival. Field research on the sea ice is also effectively limited to September through December, when the winter storms have subsided, but the sea ice is still thick enough for safe travel. Thus, if species spawn or have ontogenetic habitat shifts that are seasonally modulated at times outside of the austral summer field season, they are completely missed from observations and sampling. While logistical and seasonal constraints will continue to limit access, especially with increased sea ice instability under climate change, understanding juvenile life stages is a priority area of study.

Relying on studying only adult life stages may preclude a thorough understanding of these fishes. From an ecological perspective, we may miss important features of life history, such as reproductive strategies (i.e., when, where, and how eggs are laid) and population dynamics (i.e., recruitment and population stability) (Chan, 2018). From the physiological perspective, early life stages are thought to be more sensitive to changes in environmental conditions (Pankhurst and Munday, 2011; Pörtner and Peck, 2010), making them critical for understanding vulnerability to anthropogenic ocean change. From a behavioral perspective, early life stages are known to show distinct behaviors from their adult counterparts (e.g., habitat preference, feeding, and escape behaviors) (Gibb *et al.*, 2006; Mittelbach *et al*., 2014). Early life exposure to specific environmental conditions, through phenotypic plasticity and associated reaction norms, can produce divergent adult phenotypes, ultimately influencing their capacity to cope effectively with changing environmental conditions (Dupont *et al*., 2024; Fürtbauer *et al.*, 2015; Morita et al., 2009; Oomen and Hutchings, 2022; Travis and Trexler, 2024).

Studying juvenile life stages is critical to characterizing sensitivities that would affect the entire population.

Much of the physiological research that exists on early life stages of Antarctic fishes has been on single species, independent of their behavior and communities. Notothenioid fishes have even been grouped under one category, based on studies of *T. bernacchii*, the “generalized notothenioid” (e.g., Eastman and Lannoo, 1995). This approach is pragmatic given constraints to Antarctic fieldwork, but it implicitly assumes that all species will perform similarly under environmental conditions, which is largely untested. Given our rapidly changing world, we need a more complete picture of how communities of coexisting species differ physiologically and behaviorally under current conditions – only then can we begin to understand how interspecific variation may translate into differential capacity to tolerate environmental change.

Drivers of niche differentiation

Niche theory, the idea that every organism has a particular and distinct role within its ecosystem, has evolved over time and has ebbed and flowed in popularity in the ecological academic community (for a review see Pocheville, 2015). Across disciplines, we have different angles of approach to understanding niche differentiation; environmental physiologists tend to focus on the abiotic niche drivers, such as temperature, while ecologists tend to focus on the biotic niche drivers, such as competition and foraging (Pörtner *et al*., 2010). The biotic and the abiotic notoriously cannot be separated, so it is critical to widen our perspective of niche differentiation to integrate these factors (for examples, see Schat *et al*., 2025 and Zillig *et al.* 2021).

Contemporary ecological niche theories are valuable frameworks for studying interspecific vulnerabilities, as they integrate environmental threats (i.e., habitat destruction and climate change) and dimensions of organismal performance (i.e., thermal tolerance, metabolic rate, and mitochondrial efficiency) that may shape a species capacity to tolerate threats (Chase and Leibold, 2003; D'Andrea and Ostling, 2016; McGill *et al*., 2006; Laland *et al*., 2017; Pörtner *et al*., 2010; Scheele *et al.*, 2017; Violle and Jiang, 2009). Thus, developing a deeper understanding of the important axes that shape species niches – and their interactions – is critical for improving adaptive management strategies, especially given the rapid time scale of anthropogenic environmental change (Chase and Leibold, 2003; Godoy *et al*., 2020; Scheele *et al*., 2017). It is important to acknowledge that understanding the roles and responsibilities of every being in the ecosystem, including humans, (i.e., their niche) has been a key concept of traditional ecological knowledge for thousands of years, long before any Western scientist described niche theory (Senos *et al*., 2006).

One of the defining physiological components of an organism's niche is its metabolic rate (Ern, 2019; Gvoždík, 2018; Pörtner *et al*., 2010), as mismatches in energy supply and demand limit organismal performance (Sokolova *et al*., 2012). Routine metabolic rate (RMR) – the "most fundamental biological rate" (Brown *et al*., 2004) – is used to examine basal energy demands across species. RMR aims to describe the energy expenditure for all homeostatic processes (Pettersen *et al*., 2018) and is useful for predicting potential interspecific performance differences; if one species requires less energy to maintain homeostasis than another, it would be predicted to outperform the other when energy demands increase to cope with future

ocean conditions (Mandic *et al.*, 2009; Marquet *et al*., 2004; Sokolova *et al.*, 2012), especially in food limited ecosystems such as the Antarctic (Clarke, 1988; Pang *et al*., 2020). Fulton's condition factor (CF) can also provide insight into the energy stores of a fish, where a higher CF suggests greater energy stores (Chellappa *et al*., 1995; Herbinger and Friars, 1991). CF is also correlated with higher survival under thermal stress (Robinson *et al*., 2008). Together, RMR and CF are two important parameters that can provide insight into the energetic status of an animal.

Behavioral activities (e.g., feeding behaviors, boldness/anxiety, habitat preference) are also critical drivers of niche differentiation (Pörtner *et al*., 2010) and can be integrated with metabolic measures (i.e., RMR) to understand the physiological and behavioral trade-offs organisms must make to thrive – or just survive – in varying environmental and ecological conditions (Sokolova *et al*., 2012). Understanding such trade-offs is especially important in the context of predicting potential interspecific performance differences in the context of climate change. We used the novel object test (NOT) and novel tank test (NTT) (Cachat *et al.*, 2010; Hamilton *et al*., 2014; Hamilton *et al*., 2017), well-established assays of behavioral activity in fishes, generally, that have also been shown to be effective in juvenile Antarctic fishes to assess trade-offs between physiology and behavior (Davis *et al.* 2018). The NOT and NTT measure spontaneous swimming activity and how a fish interacts with novel situations, providing insight into the exploration-avoidance axis of fish behavior (Conrad *et al.*, 2011). Swimming activity and anxiety are thought likely to be important determinants of fitness in future oceans (Hamilton *et al.*, 2021; Rodriguez-Dominguez *et al.*, 2022; Sih *et al.*, 2011, Smith and Blumstein, 2008; Zhao and Feng, 2015), as behavior is a "key first response" to rapid

environmental changes (Sih *et al*., 2012). By examining both physiology and behavior under ecological frameworks, we gain a more comprehensive understanding of interspecific differences and can better predict potential differential performance in future oceans.

In this study we examined the baseline (i.e., no stressor exposures) behavioral and metabolic axes of niche differentiation, including RMR, CF, NOT and NTT, of four Notothenioid fishes at the juvenile life stage: *Trematomus bernacchii*, *Trematomus pennellii*, *Trematomus nicolai*, and *Pagothenia borchgrevinki*. These species were chosen because they are the most abundant juvenile notothenioids in the near-shore waters of McMurdo Sound in the Ross Sea, making them a priority of study to deepen our understanding of the ecology in this system. Of the four Notothenioid fishes represented in this study, two are currently described as benthic (*T. bernacchii* and *T. pennellii*), one epibenthic (*T. nicolai*), and one cryopelagic (*P. borchgrevinki*) at adult life stages. While *T. bernacchii* and *T. pennellii* have a varied diet, there is some evidence of food resource partitioning, which is thought to help reduce interspecific competition in a food-limited ecosystem (Barrera-Oro, 2003; Carlig *et al*., 2022; La Mesa *et al*., 2004; Pakhomov, 1997). In contrast to their benthic counterparts, *P. borchgrevinki* have a narrow diet of exclusively nektonic organisms, especially copepods and amphipods (Eastman and DeVries, 1985; Hoshiai *et al*., 1989; Janssen, 1992; Montgomery *et al*., 1989). To date, we are unaware of any published studies examining the exploratory swimming behavior of these fishes at any life stage, except for one study in juvenile *T. bernacchii* (Davis *et al*., 2018).

For over five decades, the fish physiology community has placed substantial attention on understanding how Antarctic fishes may cope with global change (for a review, see Todgham and Mandic, 2020). Much of this attention is rooted in the curiosity to understand what unique physiological adaptations underpin the ability of Antarctic organisms to survive below-freezing conditions. The present study aims to “go back to the basics” of physiological and behavioral ecology and ask how Antarctic fishes vary, generally, under current conditions. How do they differ in basal physiology early in life? Are there distinct interspecific behavioral types that could be important in understanding how these fishes may cope with global change? Ultimately, our goal is to provide some scaffolding as we build a more holistic understanding of Antarctic notothenioids and the future they may face in the rapidly changing Southern Ocean.

Materials and Methods

1. Fish Collection, Care, and Maintenance

Experiments were conducted at McMurdo Station, a National Science Foundation research station on Ross Island in McMurdo Sound, Antarctica in the austral summers of 2018 (for *Trematomus bernacchii* and *Pagothenia borchgrevinki*) and 2019 (for *Trematomus pennellii* and *Trematomus nicolai*). Fishes were collected at the intake jetty (77°51.076’S, 166°39.852’E) and Arrival Heights (77°49.906’S, 166°37.679’E); these two closely located (~1 km apart) dive sites allowed us to catch enough individuals while minimizing differences in environmental histories. Fishes were carefully collected using SCUBA with small hand-held nets and custom-designed collection bags. Fishes were transported to the aquarium room in the A.P. Crary Science and Engineering Center in

aerated coolers filled with fresh seawater within two hours of collection. Collection mortality rates were negligible.

Upon arrival at the aquarium, fishes were carefully transferred to holding tanks held at ambient temperature (approximately -1.1°C to -1.5°C), visually inspected for injuries, separated by species, and counted. We identified species based on morphological differences following a juvenile identification guide developed by AET (**Figure 1**). We assumed fishes were in their second year of age as described in previous studies (Davis *et al*., 2016; Davis *et al*., 2018; La Mesa *et al*., 1996; North, 1991). We were not able to determine sex. Animals were held for an average of five days before experimental testing to recover from collection and handling stress, but to minimize laboratory acclimation time. Fishes were fed approximately 0.03g/fish of frozen plankton (Hikari Bio-Pure Ocean Plankton, Hayward, CA, USA) twice daily during the recovery period. Human interactions with fishes (i.e., leaning over the tank) were minimized during this time. Fishes were fasted overnight prior to experiments to limit any effects of digestion. All research was conducted in accordance with US federal animal welfare laws by approval by the University of California Davis Institutional Animal Care and Use Committee (protocol no. 20558).

2. DNA Barcoding

Species identifications made via morphological characteristics were later confirmed using DNA barcoding at the University of California, Davis. DNA was isolated via GeneJet Genomic DNA purification kit (Thermo Fisher Scientific, USA) following the manufacturer protocol. Three genes were amplified: cytochrome c oxidase subunit I

(COI), NADH dehydrogenase subunit 2 (ND2), and cytochrome b (cyt b) and compared with known Antarctic fish sequences. Polymerase chain reaction (PCR) reactions were used to amplify target genes; reaction mixtures consisted of 10x reaction buffer with 20 mM $MgCl_2$, 10% trehalose, 10 µM forward primers, 10 µM reverse primers, 10 mM dNTP mix, 0.3 U Taq polymerase, and nuclease-free water. COI was amplified using using the FishF1t1 and FishR1t1 primer cocktails following Ivanova *et al*. (2007); ND2 primers were used to amplify the ND2 region following Kocher *et al.* (1995); The cyt b region was amplified using custom primers (**Appendix S. Table 1**). PCR reaction cycle protocols are reported in (**Appendix S. Table 1**). After 20 cycles of amplification, 2 uL of PCR product was removed for each gene and re-amplified. After isolation, PCR products were visualized on a 1.5% agarose gel (BioRad ChemiDoc MP Imager, USA) and purified using the GeneJet PCR Purification Kit (Thermo Fisher Scientific, USA) following the manufacturer protocol. After purification of PCR products for each gene, samples were sequenced with 5uM of the respective forward primer for each gene, with the addition of the M13F(-21) primer to sequence the COI region (Messing, 1983; Ivanova *et al.*, 2007). COI, ND2, and cyt b gene sequences were checked for mismatched nucleotides (≤1 mismatches) and identified via the Basic Local Alignment Search Tool (BLAST) in the National Center for Biotechnology Information (NCBI).

3. Metabolic Rate

Routine metabolic rate (RMR) was calculated using oxygen consumption measurements from intermittent respirometry. Two trials of eight fish, or four trials of four fish in the case of *T. pennellii*, were conducted during daytime hours in a cold-storage

room held at -2°C. Due to a handful of anomalous oxygen consumption slopes during experiments – due to isolated equipment malfunction – we measured an additional three *T. nicolai* and *T. bernacchii* for a total *N*=70. Lights were kept off, and access was limited to only the experimenter in order to minimize disturbance of the fish. For each trial, fish were gently netted from holding tanks and carefully carried to the cold room in a bucket with fresh seawater. Fish were then quickly and gently transferred into respirometers using a small aquarium net with minimal air immersion time. Trials began after all fish were placed in their respirometers.

Fish were held in respirometers for six measurement cycles consisting of a 10-minute flush, 5-minute wait, and 20-minute measurement period, totaling 3.5 hours. Oxygen saturation never fell below 80%, as recommended in Svendsen *et al*. (2016). Measurement cycles were controlled using automated software (AutoResp v2.3.0, Loligo Systems, DK) and two 8-channel low-flow peristaltic pumps – one pump was used to control the recirculating flow and the other pump was used to control the flush (Model BT100-1L, Langer Instruments, USA). Conducting the respirometry measurements in the cold-room allowed for nearly perfect temperature regulation, preventing any air bubbles forming in the tubing. Oxygen consumption was measured using fiber optic probes connected to two 4-channel oxygen meters (Witrox 4, Loligo Systems, DK) and data acquisition equipment (DAQ-M, Loligo Systems, DK). The fiber optic probes were aligned with optical oxygen sensor spots (PreSens, DE) that were fixed to the inside of the chamber wall with silicone glue. Probes were calibrated with 100% oxygenated seawater the day before trials.

The chambers consisted of a custom-made glass cylinder with a rubber stopper on each end pierced with two stainless steel pipes to connect to the flow tubing. Each respirometer was completely independent from the others and had a high-grade gas impermeable silicone tubing line (Tygon E-3603, Saint-Gobain, FR) running through each peristaltic pump. *T. bernacchii*, *T. pennellii*, *T. nicolai*, and *P. borchgrevinki* were measured in 26, 15.7, 33.4, and 26 mL chambers, respectively, to ensure that the fish weight to water volume ratios were from 1% to 5% as described in Clark *et al*. (2013). Fresh, oxygenated seawater held in coolers was used to flush the respirometry chambers. Respirometers were fully submerged in seawater baths to maintain ambient temperatures.

After each trial of *T. bernacchii* and *T. borchgrevinki*, we ran a trial with empty chambers to measure any background respiration. For *T. pennellii* and *T. nicolai*, we stopped running a blank trial after each trial because we found extremely low background respiration with very low variability between trials and chambers. Instead, we ran one blank trial after the final trial of fish for the day. After the final trials were completed for the day, we cleaned the respirometers by thoroughly rinsing with DI water and gently scrubbing the glass with Kimwipes wrapped around cotton swabs; we were careful not to touch the oxygen sensor spot. The tubing was also flushed with DI water and the bath was thoroughly rinsed. All equipment was allowed to completely dry between days.

Metabolic rate was calculated in R using the R Studio interface (v4.4.1, R Core Team, 2024). Test trials consistently showed reduced and stabilized metabolic rate after the first two cycles, indicating that these fishes recovered from the acute handling stress

within about an hour; accordingly, the first two cycles were removed and the lowest three measurement periods thereafter were then averaged. Metabolic rate was then corrected for background respiration; we calculated one average blank value for each species – because of 1) the low variation in background respiration across chambers and trials and 2) species being measured in differently sized respirometry chambers – as the average of multiple blanks. We removed two *T. bernacchii*, one *P. borchgrevinki*, and four *T. nicolai* metabolic rates (including the three that we observed as anomalous during the trials) due to R^2 values falling under 75% (the average R^2 of retained metabolic rates was 96%). Accordingly, final N=60 (n=14 for *T. bernacchii*, n=16 for *T. pennellii*, n=15 for *T. nicolai*, and n=15 for *P. borchgrevinki*). The reported values are the blank-corrected, mass-specific routine metabolic rates for each species.

4. Behavioral Activity

Novel Object Tests were performed following a modified protocol from Hamilton *et al.* (2014) and Hamilton *et al.* (2017). Circular buckets (20 cm diameter) with 1 x 1 cm grids affixed to the bottom were filled with 6 cm seawater and placed in a water bath to maintain ambient temperature. Individuals were gently and quickly transferred to the arenas and lids with affixed action cameras (Activeon CX Action Camera, United States) were placed on top of the arena. Baseline fish behavior was recorded for ten minutes. Subsequently, the lids were gently removed, novel objects were carefully placed into the center of the arena, and lids were replaced. LEGO ‘Female Wildlife Photographer’ Minifigures were used as the novel object as they were an appropriate size compared to the fishes, displayed colors across the spectrum, and included complex shapes. Fish

behavior with the novel object was then recorded for ten minutes. Four fish were trialed concurrently. Twenty individuals were measured per species.

Novel Tank Tests were performed following a modified protocol from Cachat *et al.* (2010). Trapezoidal tanks (14.4 L) with back, bottom, and side grids were filled to 18 cm with fresh seawater and placed in a water bath to maintain ambient temperature. Individuals were gently transferred into the tanks and translucent plastic sheets were tented over the testing area to minimize external disturbance. Fish behavior was recorded for eighteen minutes with a side-view action camera submerged in the water bath. Experimenters left the aquarium when experiments were running and three fish were run concurrently. Twenty individuals were measured for each species, except for *T. nicolai* which had nineteen individuals.

All video recordings were analyzed using EthoVisionXT v17.4 (Noldus, NL) video tracking software. For the NOT, we used QuickTime Player to split each video into halves, the first half being the control and the second half with the novel object. A unique arena was designated for every video half due to slight variations in camera angles from observers replacing the lids following placement of the novel object. The arenas were designated as the Wall, Outer, Middle, and Center zones and the novel object was marked as space impossible for the fish to occupy **(Appendix S. Figure 1)**. For the NTT, a unique arena was designated for every individual. The arenas were designated into equal thirds of Top, Middle, and Bottom zones **(Appendix S. Figure 1)**. For both the NOT and NTT, we tracked the center-point of the fish using contour-based detection. We set the zone exit threshold to 1.0 cm. For NOT, the first ten seconds were removed; we did not see any patterns in movement over the time of the trial to suggest

that removing more time was necessary for the fishes to settle. For NTT, the track was started 10 seconds after the water movement completely settled, approximately two minutes after the trial started.

After video acquisition, we watched every recorded trace to ensure the fish was accurately tracked. When tracks were poor (i.e., when the fish was inaccurately tracked more than ~25% of the trial time), we reran the acquisition of that trial with unique detection settings optimized for that specific fish. When tracks had minor missing segments or the software identified something other than the fish as the fish, we manually fixed these errors using the Interpolate tool and by dragging the Center Point dot to the correct location on the fish. Statistical outliers were not removed, for a final N=80 total for all species for the NOT. For the NTT, one video (*T. nicolai*) was lost as the camera battery failed during the trial, for a final N=78 for all species.

Metrics of interest for the NOT and NTT were selected *a priori*, based on previous literature and the known behavioral ecology of these species, as well as *posteriori* via Principal Component Analysis (PCA). The PCAs were conducted in R using the R Studio interface. We generated normalized data and principal components using “stats” and visualized the PCA using loadings order, scree plots, biplots, and cos2 order using the “factoextra” and “FactoMineR” packages (Kassambara and Mundt, 2020; Le *et al.*, 2008). For the NOT, the variables selected were the mean velocity, maximum velocity, frequency of stop-starts, mean distance to center point, frequency entering the center zone, frequency entering the outer zone, and frequency entering the wall zone. For the NTT, the most descriptive variables selected were the mean and maximum velocity, frequency entering the top and bottom zones, maximum time spent

in the top and bottom zones, and the cumulative duration spent in the top and bottom zones.

We used mean velocity and maximum velocity to understand general swimming capacity and as insight into energy spent on swimming. We used the frequency of stop-starts to examine the juvenile fishes swimming ecotypes and to understand if they are consistent with the swimming ecotypes of the adult counterparts. The frequency of entering zones and the time spent in zones are indicators of exploration vs. avoidance (i.e., anxiety), where staying in the outer zone for the NOT or the bottom zone for the NTT indicate heightened anxiety (Maximino *et al*., 2010). Fish entering/exiting zones can also be an indication for general exploratory activity.

5. Morphometrics

Morphometrics measured include weight, standard length, and Fulton's condition factor (CF). CF was calculated as:

$$Condition\ Factor\ = \frac{Weight\ (g)}{Total\ Length\ (cm)^3} \times 100$$

After RMR and behavior trials, fish were immediately sacrificed in a lethal dose of tricaine methanesulfonate (500 mg/L, MS-222) prepared in seawater. Fish were then gently dabbed dry, immediately weighed to the nearest 0.01 g, and the standard length measured using calipers to the nearest 0.1 mm. Morphometric data include all individuals across RMR and behavior experiments, minus three individuals with missing morphometric data.

6. Statistical Analyses

All statistical analyses were conducted in R using the R Studio interface. Analyses were conducted following a mixed effects framework using linear mixed effects models via “nlme” or, for frequency datasets, using generalized linear mixed effects models with a negative binomial distribution via “glmmTMB” (Brooks *et al*., 2017; Pinheiro and Bates, 2000; Pinheiro *et al*., 2023). We also used a generalized linear mixed effects model for maximum velocity using “lme4” to specify a gamma distribution (Bates *et al*., 2015). Models were inspected for linearity, via visual inspection of fitted values vs. residuals; homoscedasticity, via the Breusch-Pagan test in the “lmtest” package and visual inspection of fitted values vs. residuals (Zeileis and Hothorn, 2002); independence, via visual inspection of lag plots; and normality, via visual inspection of QQ plots and histogram of model residuals, as advised in Zuur *et al*. (2010). If a model showed heteroscedasticity, weights were added to allow for a different variance parameter for each species. Models were built via biologically informed backwards model selection as advised in Harrison *et al*. (2018) and Zuur *et al.* (2009). The random structure was retained in all models regardless of significance of random effects predictors to retain the experimental design in the models. For morphometric models, we used ‘experiment’ (i.e., if the morphometric data was sourced from individuals used in RMR, NOT, or NTT) as a random effect. For RMR, we modeled ‘respirometry chamber’ as a random effect. For NOT models, ‘fish ID’ and ‘bucket’ were included as random effects. Finally, for NTT datasets, ‘tank’ was included as a random effect. To determine the fixed effects structure models, we evaluated nested models against the reference model using maximum likelihood testing via an ANOVA (via “stats”). Parameters that were tested for

removal were weight and length. We compared the model fits via AIC, BIC, log-likelihood, and p-values (**Appendix S. Table 2**) and dropped weight and/or length from the model if appropriate. For all models, species was always retained in the model regardless of significance. For NOT models, 'novel object' ('pre', before the object was added; 'post', after the object was added) was always included as an interactive effect with species. The best-fit model summaries are available in **Appendix S. Table 3.** For all models, the "emmeans" package was used to calculate pairwise comparisons of means across species, without adjustment (Lenth, 2024). We adjusted p-values for false discovery rate for each set of models belonging to the same experiment using the Benjamini-Hochberg correction (via "stats"). All contrasts are available in **Appendix S. Table 4.** An α of 0.05 was used as the significance level for all statistical tests. The reported estimates for all frequency and percentage data are on the log scale and logit scale, respectively, not the response scale. We calculated the conditional and marginal R^2 for each model to evaluate model fit and variance explained by fixed vs. random effects via the "MuMIn" and "performance" packages (Bartón, 2024; Lüdecke *et al*., 2021), as recommended in Nakagawa and Schielzeth (2013). For NOT models, we also calculated the Intra-class coefficients (ICC) for each model (via "performance") to understand the degree of repeatability of individuals. Data visualization was conducted using the "ggplot2" package (Wickham, 2016) and figure design in Inkscape (v1.3.2, Inkscape Team, 2023).

Results

1. Morphometrics

Weight

Species' weights were modeled with a linear model with species as the fixed effect, experiment as the random effect, and model weights for Species. Species explained approximately 65% of the variance in weight (marginal $R^2 = 0.65$) and there was a minor effect of experiment (conditional $R^2 = 0.66$). Weights of all species differed significantly from one another (all $p < 0.0001$; **Appendix S. Table 5**), where *T. nicolai* were the heaviest (mean ± SD: 1.35 ± 0.28 g), followed by *P. borchgrevinki* (0.70 ± 0.16 g), then by *T. pennellii* (0.54 ± 0.10 g), and then finally by *T. bernacchii* (0.33 ± 0.04 g) (**Figure 2**). *T. bernacchii* weighed 0.21 g less than *T. pennellii*, 1.01 g less than *T. nicolai*, and 0.37 g less than *P. borchgrevinki* (all $p < 0.0001$).

Standard Length

Standard lengths were also modeled with a linear model with species as the fixed effect, experiment as the random effect, and model weights for species. Species explained approximately 84% of the variability in length (marginal $R^2 = 0.84$) with experiment providing no additional explanation (conditional $R^2 = 0.84$). Standard lengths of all species differed significantly from one another (all $p < 0.0001$), where *T. nicolai* were the longest (mean ± SD: 48.29 ± 2.26 mm), followed by *P. borchgrevinki* (42.90 ± 2.30 mm), then by *T. pennellii* (39.12 ± 1.67 mm), then by *T. bernacchii* (34.19 ± 3.52 mm) (**Figure 2**).

Condition Factor

Condition factor (CF) was also modeled with a linear model with species as the fixed effect, experiment as the random effect, and model weights for species. Species explained over half of the variance in CF (conditional R^2 = 0.59) and there was some effect of experiment (marginal R^2 = 0.61). *T. nicolai* (mean ± SD: 1.18 ± 0.11) had a significantly higher CF than *T. bernacchii* (0.91 ± 0.44; est = -0.27, $p < 0.0001$), *T. pennellii* (0.90 ± 0.08; est = -0.29, $p < 0.0001$), and *P. borchgrevinki* (0.87 ± 0.09; est = 0.31, $p < 0.0001$) (**Figure 2**), but *T. bernacchii*, *T. pennellii*, and *P. borchgrevinki* did not differ significantly (all $p > 0.17$). When species were modeled individually, we found a strong relationship between weight and length for *T. pennellii*, *T. nicolai*, and *P. borchgrevinki* (adjusted R^2 = 0.82, 0.86, 0.88, respectively) and no relationship between weight and length for *T. bernacchii* (adjusted R^2 = -0.02) (**Appendix S. Figure 2 and S. Table 5**). Accordingly, *T. bernacchii* had a much larger variation in CF than the other three species.

2. Metabolic Rate

Our best fit model of routine metabolic rate (RMR) was a linear model with species and length as the fixed effects, chamber as the random effect, weighted by the variance for each species. Species only explained approximately 15% of the variance in RMR (marginal R^2 = 0.14), and while the model indicated an overall effect of species on RMR, after post-hoc correction, pairwise comparisons were no longer statistically significant (all p > 0.08) (**Figure 3**). The explained variance approximately doubled with the

random effects (conditional R^2 = 0.37), indicating that a considerable portion of the variance was explained by respirometry chambers.

3. Novel Object Test

Mean Velocity

Our best fit model of mean velocity was a linear mixed effects model with the interaction of species and novel object, weight, and length as fixed effects and fish ID and bucket as random effects. The combined fixed effects in our model explained 20% of the variance in velocity (marginal R^2 = 0.20), and with random effects nearly 90% of the variance was explained (conditional R^2 = 0.88). *T. bernacchii*, *T. pennellii*, and *P. borchgrevinki* significantly decreased their velocity after the novel object was added (Δ=0.57 cm/s, Δ = 0.95 cm/s, Δ = 0.50 cm/s, respectively; all $p < 0.001$), while *T. nicolai* showed no significant change (Δ = 0.12 cm/s, p = 0.39) (**Figure 4**). There were no significant interspecific differences in mean velocity before or after the novel object was added (all $p > 0.12$).

Maximum Velocity

Our best fit model of maximum velocity was a generalized linear mixed effects model with the interaction of species and novel object as fixed effects and fish ID and bucket as random effects. The combined fixed effects and random effects did not explain variance in the data (marginal and conditional $R^2 < 0.01$) indicating other factors are likely driving the variation in maximum velocity. The adjusted ICC was 0.004, showing no correlation between maximum speed swum before and after the novel object was

added. *T. nicolai* significantly increased their maximum velocity after the novel object was added (1.6% change, $p = 0.05$) (**Figure 4**). There were no significant interspecific differences in maximum velocity before the novel object (all $p > 0.12$), but after the novel object was added, *T. pennellii* and *T. nicolai* had significantly higher maximum velocity than *T. bernacchii* (3% change, $p = 0.05$; 4% change, $p = 0.04$, respectively). *T. nicolai* also had significantly higher post- novel object maximum velocity than *P. borchgrevinki* (4% change, $p = 0.04$), and *T. pennellii* showed a similar trend (3% change, $p = 0.06$). All other pairwise comparisons were not significant ($p > 0.89$).

Frequency of Stop-Starts

Our best fit model of the frequency of stop-starts was a linear mixed effects model with the interaction of species and novel object as fixed effects and fish ID and bucket as random effects. The combined fixed effects in our model explained nearly a third of the variance in stop-starts (marginal $R^2 = 0.32$), and with the random effects the explained variance increased to nearly 75% (conditional $R^2 = 0.73$). Only *P. borchgrevinki* significantly increased their frequency of stop-starts after the novel object was introduced (27% increase, $p = 0.05$) (**Figure 4**). For interspecific comparisons, *T. nicolai* stop/start swam significantly more than *T. bernacchii*, *T. pennellii*, and *P. borchgrevinki* both before and after the novel object was added (all $p < 0.001$). *T. bernacchii*, *T. pennellii*, and *P. borchgrevinki* did not significantly differ in their stop/start swimming (all $p > 0.20$).

Mean Distance to Center Point

Our best fit model of the mean distance to the center point was a linear mixed effects model with the interaction of species and novel object as fixed effects, fish ID and bucket as random effects, weighted by the variance for each species. The combined fixed effects in our model explained a quarter of the variance in distance (marginal R^2 = 0.25), and with the random effects the explained variance increased to three quarters (conditional R^2 = 0.76). *T. pennellii* and *P. borchgrevinki* significantly decreased their distance to the center point (i.e., distance to the novel object) after the novel object was added (Δ = 0.78 cm, p = 0.006; Δ = 0.59 cm, p = 0.001, respectively), while *T. bernacchii* and *T. nicolai* showed no significant change (p = 0.14 and 0.87, respectively) (**Figure 4**). For interspecific comparisons, *T. bernacchii*, *T. pennellii*, and *T. nicolai* went significantly closer to the center point than *P. borchgrevinki* before the novel object was added (est = -0.66 cm, p = 0.02; est = -0.77 cm, p = 0.02; est = -0.58 cm, p = 0.04, respectively) (**Figure 4**). After the novel object was added, *T. pennellii* moved significantly closer to the novel object than *T. bernacchii* (est = 0.68 cm, p = 0.03), *T. nicolai* (est = -0.92 cm, p = 0.006), and *P. borchgrevinki* (est = -0.95 cm, p = 0.002) (**Figure 4**). All other pre- and post- object interspecific comparisons were non-significant (p > 0.38).

Frequency Entering Center Zone

Our best fit model of the frequency of entering the center zone was a generalized linear mixed effects model, with a negative binomial distribution, with the interaction of species and novel object as fixed effects and fish ID and bucket as random effects. The

combined fixed effects in our model explained a quarter of the variance in frequency (marginal R^2 = 0.25), but with the random effects the explained variance was half (conditional R^2 = 0.49). The adjusted ICC was 0.39, indicating low repeatability in how many times individual fishes entered the center zone. *T. bernacchii* and *T. nicolai* significantly decreased their frequency of entering the center zone after the novel object was added (38%, p = 0.02; 32%, p = 0.05, respectively), while *P. borchgrevinki* significantly increased theirs (65%, p = 0.04) (**Figure 4**). *T. pennellii* did not significantly change their frequency of entering the center zone after the novel object was added (p = 0.83). Before the novel object was introduced, *P. borchgrevinki* entered the center zone significantly less frequently than *T. bernacchii* (66%, p = 0.001), *T. pennellii* (66%, p = 0.001) and *T. nicolai* (71%, p = 0.001). After the novel object was added, only *T. pennellii* and *P. borchgrevinki* significantly differed in their frequencies of entering the center zone, with *T. pennellii* entering 66% more often (p = 0.05). All other pre- and post- novel object interspecific comparisons were non-significant (all p > 0.12).

Frequency Entering Outer Zone

Our best fit model of the frequency of entering the outer zone was a generalized linear mixed effects model, with a negative binomial distribution, with the interaction of species and novel object as fixed effects and fish ID and bucket as random effects. The combined fixed effects in our model explained less than a quarter of the variance in frequency (marginal R^2 = 0.14), but with the random effects about half of the variance was explained (conditional R^2 = 0.49). The adjusted ICC was 0.44, indicating low repeatability in the frequency that individual fishes entered the outer zone. After the

novel object was introduced, *T. bernacchii* entered the outer zone 36% less often (p = 0.002) (**Figure 4**). No other species showed a difference in visits to the outer zone pre- versus post- novel object (all $p > 0.14$). For interspecific comparisons, *T. pennellii* entered the outer zone 58% more often than *P. borchgrevinki* before the novel object was added (p = 0.02). After the novel object was added, *T. pennellii* entered the outer zone 47% more often than *T. bernacchii* (p = 0.05) and 51% more often than *P. borchgrevinki* (p = 0.04). All other pre- and post- novel object interspecific comparisons were non-significant (all $p > 0.14$).

Frequency Entering Wall Zone

Our best fit model of the frequency of entering the wall zone was a generalized linear mixed effects model, with a negative binomial distribution, with the interaction of species and novel object as fixed effects and fish ID and bucket as random effects. The combined fixed effects in our model explained less than a quarter of the variance in frequency (marginal R^2 = 0.19), but with the random effects over half of the variance was explained (conditional R^2 = 0.59). The adjusted ICC was 0.49, indicating low repeatability in the frequency that individual fishes entered the wall zone. All species significantly reduced their frequency of entering the wall zone after the novel object was added (*T. bernacchii*: 41%, p = 0.01; *T. pennellii:* 53%, p = 0.001; *T. nicolai:* 36%, p = 0.03; *P. borchgrevinki*: 39%, p = 0.01; **Figure 4**). There were no significant interspecific differences in frequency of entering the wall zone before or after the novel object was added (all $p > 0.12$).

4. Novel Tank Test

Mean Velocity

Our best fit model of mean velocity was a linear mixed effects model with Species as the fixed effect, tank as the random effect, and variance weights for each species. Our model did not explain variance in mean velocity (marginal R^2 = 0.03, conditional R^2 = 0.03). We found no significant differences in mean velocity in any contrast of species (all p > 0.63).

Frequency Entering Top Zone

Our best fit model of the frequency of entering the top zone was a linear mixed effects model with species as the fixed effect and tank as the random effect. Species explained a small proportion of variance in the frequency of entering the top zone (marginal R^2=0.13) with tank providing no additional explanation (conditional R^2=0.13). *T. pennellii* entered the top zone 45% less often than *P. borchgrevinki* (p = 0.03), but all other contrasts were nonsignificant (all p > 0.08; **Figure 5**). There was an apparent bimodal distribution within *T. nicolai*, with about half of the individuals visiting the top zone frequently and the other half rarely visiting the top zone.

Frequency Entering Bottom Zone

Our best fit model of the frequency of entering the bottom zone was a linear mixed effects model with species as the fixed effect and tank as the random effect. Species explained a small proportion of variance in the frequency of entering the bottom zone (marginal R^2 = 0.11) with tank providing no additional explanation (conditional R^2 = 0.11).

T. pennellii entered the bottom zone 42% less often than *T. bernacchii* (p = 0.03) and 48% less often than *P. borchgrevinki* ($p < 0.01$) (**Figure 5**). All other contrasts were nonsignificant (all $p > 0.10$). All species entered the bottom zone with about the same frequency as they entered the top zone.

Maximum Time in Top Zone

Our best fit model of the maximum time spent in the top zone was a linear mixed effects model with species as the fixed effect, tank as the random effect, and variance weights for each species. Species explained about a third of the variance in the maximum time in the top zone (marginal $R^2 = 0.33$) with tank providing no additional explanation (conditional $R^2 = 0.33$). *T. bernacchii* and *T. pennellii* both spent longer visits in the top zone than *T. nicolai* (est = 24.9 and 29.8 s, respectively; $p < 0.001$ for both) and *P. borchgrevinki* (est = 17.4 and 22.3 s, respectively; $p < 0.001$ for both).

Maximum Time in Bottom Zone

Our best fit model of the maximum time spent in the bottom zone was a linear mixed effects model with species as the fixed effect, tank as the random effect, and variance weights for each species. Species explained about a quarter of the variance in maximum time in the bottom zone (marginal $R^2 = 0.26$) with tank providing minor additional explanation (conditional $R^2 = 0.27$). *T. bernacchii* had significantly shorter visits in the bottom zone than *T. nicolai* and *P. borchgrevink* (est = -0.77 and -0.54 s, respectively; $p < 0.02$ for both). They also spent shorter visits in the bottom zone than *T. pennellii*, but that difference was nonsignificant (est = -0.50, $p = 0.06$).

Percent Time in Top Zone

Our best fit model of percent of time spent in the top zone was a linear mixed effects model with species as the fixed effects and tank as the random effect. Species explained over half of the variance in percent time in the top zone (marginal R^2 = 0.55) with tank providing no additional explanation (conditional R^2 = 0.55). *T. nicolai* spent significantly less time in the top zone than *T. bernacchii* (est = 1.9%, p = 0.001), *T. pennellii* (est = 1.8%, p = 0.001), and *P. borchgrevinki* (est = -1.4%, p = 0.001). All contrasts between *T. bernacchii*, *T. pennellii*, and *P. borchgrevinki* were nonsignificant (all p > 0.09).

Percent Time in Bottom Zone

Our best fit model of percent of time spent in the bottom zone was a linear mixed effects model with species as the fixed effect and tank as the random effect. Species explained almost two thirds of the variance in percent time in the bottom zone (marginal R^2 = 0.63) with tank providing no additional explanation (conditional R^2 = 0.63). *T. bernacchii* and *T. pennellii* spent significantly less time in the bottom zone than *T. nicolai* (est = 0.69%, p = 0.005; and 0.58%, p = 0.02, respectively) and *P. borchgrevinki* (est = 0.65%, p = 0.005; est = 0.55%, p = 0.02, respectively).

Discussion

In this study we used a comparative approach, within a framework grounded in niche theory, to investigate variation in basal physiological and behavioral traits of juvenile

Notothenioid fishes. We focused on traits important for fitness under expected future ocean conditions, such as routine metabolic rate – “the most fundamental biological rate” – and swimming behavior – a “key first response” to rapid environmental changes (Brown *et al*., 2004; Sih *et al*., 2012). Organismal level traits are also thought to be the most important for shaping a species’ niche (Pörtner *et al.*, 2010) and are therefore critical to understanding larger ecosystem dynamics such as community composition. A better understanding of baseline differences between species – especially differences thought to be important in species fitness and niche differentiation – will be critical to predicting how species, communities, and ecosystems will respond to rapidly amplifying environmental threats.

Energetic status

According to trait-based niche theory, species that share habitat and resources at the same life stage are expected to exhibit convergent physiological traits that optimize survival under overlapping selective pressures (McGill *et al*., 2006; Sutton *et al.*, 2021; Violle and Jiang, 2009). Given that, as juveniles, these species school together and occupy a shared ecotype within the same environment, they would be expected to also experience analagous metabolic demands, despite known differentiation in adult ecotypes. Our results align with this expectation. All four species had very similar basal metabolic demands, evident in their comparable routine metabolic rates and condition factor (CF) at the juvenile life stage (except for higher CF in *T. nicolai*). Their metabolic demands may be evolutionarily conserved, which could be due to strong selection pressure resulting from the extreme conditions of the Antarctic environment, such as its

frigid waters, high UV radiation, iceberg scouring, and strong seasonality of light and – consequently – food resources (Bilyk *et al*., 2023; Burton *et al*., 2011). The idea of conserved metabolic demands is also supported by the comparable mean swimming velocity among species, evident in the lack of significant interspecific differences in either the Novel Object or Novel Tank Tests. Given that swimming is a significant energetic cost in fishes (Pang, 2020; Videler, 1993), the similar mean velocities suggest that these fishes may also share analagous energy expenditures in addition to sharing baseline energy requirements.

As adults, *T. bernacchii*, *T. pennellii*, and *T. nicolai* are benthic or epi-benthic; they sit-and-wait to feed (Hubold *et al*., 1991; Montgomery *et al*., 1993) and it is thought that their slow pace of life helps them conserve energy and survive in a frigid environment (Peck, 2018; Peck *et al.*, 2006; Pörtner *et al*., 2007). *P. borchgrevinki*, however, are cryopelagic and are thought to spend much more time actively swimming as adults. In the context of adult ecotypes, it is therefore somewhat unexpected that *P. borchgrevinki* did not exhibit higher mean velocity than the other species. At the juvenile life stage, however, these species are found schooling together in the shallows near the sea-ice where, for the most part, adults are not found. The equivalent mean velocities suggest that at the juvenile life stage, these four species have not yet differentiated into their ecotypes. It is plausible that as juveniles, these fishes share the similar energetic demands of growing and developing in a harsh environment, and consequently undergo niche differentiation through ontogeny.

The main difference that we found in the energetic status across species was in a higher condition factor (CF) of *Trematomus nicolai*. A higher CF suggests *T. nicolai*

may have greater on-board energy stores, which could give them an advantage in a "feast or famine" ecosystem such as the Antarctic (Clarke, 1988). There are no available direct comparisons of CF in adults of the four study species, but a recent report of fishes on the Antarctic peninsula found that *T. bernacchii* had poor condition in their biotypes compared to five other species (Kim *et al.*, 2023). CF is correlated with lipid content (Costopoulos and Fonds, 1989; Hards *et al.*, 2019; Herbinger and Friars, 1991; Mozsár *et al*., 2015); previous studies have found that another epibenthic Antarctic species (*Trematomus lepidorhinus*) had intermediate levels of lipid content when compared to pelagic species (*P. borchgrevinki*, *Pseudomonas antarcticum* and *Dissostichus mawsoni*) and benthic species (*Bathydraco marri* and *Dolloiodraco longedorsalis*), which had highest and lowest levels of lipids, respectively (Burns *et al*., 1998; Friedrich and Hagen, 1994). Given the higher lipid content found in other pelagic Antarctic fishes, it is somewhat surprising that we did not find *P. borchgrevinki*, the only cryopelagic species in our study, to have a higher CF. Further studies examining the energy budgets of these fishes – ideally across ontogeny – such as via proximate composition analysis, aerobic scope, cellular metabolic enzymes, and/or mitochondrial respiration, are needed to understand if the difference in CF is representative of a larger energy budget overall.

We found some evidence of differential swimming capacity, via maximum swimming velocities, where *T. pennellii* and *T. nicolai* had significantly higher maximum velocities than *T. bernacchii* and *P. borchgrevinki* after the novel object was added. While this is not as direct a measure of maximal swimming capacity as critical swimming speed (U_{crit}), these data suggest that *T. pennellii* and *T. nicolai* may have

higher swimming capacity. Faster swimming capacity could be related to a larger aerobic scope (i.e., more energy available). Swimming capacity is thought to be an important driver of fitness in fishes; capacity for faster swimming may aid in feeding, finding a mate, and avoiding predation or other threats (Plaut, 2001; Videler, 1993). There is some evidence that *T. bernacchii* and *T. pennellii* may have commensurate cardiac metabolic capacity, as indicated by comparable levels of mitochondrial function of permeabilized cardiac fibers (Mandic *et al.*, 2022), suggesting similarities in metabolic function between these two species. This leads to the question of why *T. bernacchii* did not exhibit comparable maximum velocity as *T. pennellii* when they have similar cardiac metabolic capacity. The high variability in condition of *T. bernacchii* indicates that something other than metabolic cardiac capacity limits their swimming capacity – potentially lower muscle mitochondrial density, differing muscle fiber composition, or less efficient oxygen extraction at the gills. Additional studies, such as aerobic scope and U_{crit} studies are needed to better understand the drivers of differential swimming capacities of these fishes.

A conserved freezing strategy?

While these species shared a similar energetic status, they generally exhibited divergent behavioral profiles. As aforementioned, these fishes school together at this life stage, indicating some level of a shared behavioral strategy. Under a niche theory framework, however, it would be expected that each species would vary enough in their behavior to ensure that they are all fed while also avoiding predation. Our results are consistent with this expectation.

In the NOT, *T. bernacchii*, *T. pennellii*, and *P. borchgrevinki* all significantly reduced their mean velocity after the novel object was placed, indicating a potentially conserved freezing response to novel encounters, wherein they reduce activity to avoid drawing attention (Maximino *et al*., 2010). This behavior could also be related to a sit-and-wait feeding strategy, which has been observed in adults of these fishes (Hubold *et al*., 1991; Montgomery *et al*., 1993; pers. obs.). *T. bernacchii* entered the outer zone less frequently after the object was placed – the other three species showed this trend but did not show a significant decrease – which further supports a freezing response, because the fishes are reducing activity rather than seeking refuge in the safer outer zone. At the juvenile life stage, divers consistently observe these fishes retreating into the platelet ice structure when disturbed, potentially to avoid predation (pers. obs.). At the juvenile life stage, these fishes are highly translucent and, anecdotally, have been observed to modulate their pigmentation (Davis *et al*., 2018), suggesting that camouflage in the ice may be a predation avoidance adaptation, similar to the ice camouflage observed in adult *P. borchgrevinki* (Eastman and DeVries, 1985).

Divergent exploratory behavior

We found that *T. pennellii* and *P. borchgrevinki* move significantly closer to the center of the arena after the novel object was placed, suggesting higher interest in the novel object as compared to *T. bernacchii* and *T. nicolai*, which did not move any closer to the center. After the object was placed, however, *T. nicolai* entered the center zone more often, *P. borchgrevinki* entered less often, and *T. bernacchii* and *T. pennellii* exhibited no change, demonstrating opposing strategies when faced with novelty. *T. pennellii* may be

interested and risky, as they get closer to the object and leave the center zone less frequently, whereas *P. borchgrevinki* may be interested yet cautious, as they also swim closer to the object but enter the center zone more frequently, meaning they crossed back into the “safer” zones more often. This could be an indication of predator inspection behavior, but predator-prey experiments are needed to test this observation.

In the NTT, all species entered the top zone and bottom zone at consistent rates, suggesting that the frequency of entering zones is more tied to exploratory behavior than a preference for one area of the tank. *T. bernacchii* and *T. nicolai* showed comparable exploratory activity in both the NOT and NTT, but we found differences in exploratory activity in *T. pennellii* and *P. borchgrevinki*. In the NOT, *P. borchgrevinki* entered the center zone less frequently than *T. pennellii* and *T. nicolai* before the object was placed, suggesting lower exploratory behavior of the benthic (horizontal) axis of space in the cryopelagic species. However, in the NOT *P. borchgrevinki* entered the wall zone significantly more than *T. pennellii* and in the NTT, *P. borchgrevinki* entered both the top and bottom zones more frequently than *T. pennellii*, suggesting higher exploratory behavior of the pelagic (vertical) axis of space in the cryopelagic species. In the NOT, *T. pennellii* spent the least amount of time in the outer zone and the most amount of time in the center zone across the species; in the NTT, *T. pennellii* entered the bottom zone fewer times than *T. bernacchii*. Taken together, these results indicate that *T. pennellii* may have a particularly risky strategy in novel situations. In the NTT, *T. bernacchii* and *T. pennellii* spent the longest visits in the top zone, again suggesting more exploratory, riskier behavior. *P. borchgrevinki* spent comparable cumulative amount of time in the top zone as *T. bernacchii* and *T. pennellii*, but, as seen in the NOT,

P. borchgrevinki entered/exited the zones more, suggesting cautious interest, as they swim back into the “safer” bottom zone more frequently. *T. nicolai* spent both the longest visits in the bottom and the longest cumulative duration of time in the bottom zone. Taken together, our results suggest that *T. bernacchii* and *T. pennellii* are comparatively more exploratory and riskier, *T. nicolai* is more avoidant, and *P. borchgrevinki* are exploratory, but cautious.

It is somewhat unexpected that *T. nicolai* showed the most avoidant behavior; it has been demonstrated that bolder fishes can have higher condition (Brown *et al*., 2007*a*; Luttbeg and Sih, 2010; Pellegrini *et al*., 2010) and it is thought this is either because ‘stronger’ fishes are better able to cope with predators or because bold individuals may have higher access to food (Sih *et al.*, 2004*a*). Given the higher condition found in the more avoidant juvenile, *T. nicolai*, it is unclear if this theory applies to Antarctic fishes. More research is needed to understand if boldness is adaptive in the Antarctic ecosystem; perhaps a sit-and-wait strategy, documented in adult Antarctic fishes, outweighs boldness in such an extreme and energy limited system (Hubold *et al*., 1991; Montgomery *et al*., 1993).

Intraspecific variation and behavioral syndromes

While the present study is focused on interspecific differences, we found notable intraspecific variation across physiological and behavioral traits. This was first apparent in the morphometric data, where *T. nicolai* and *P. borchgrevinki* had higher variability in weight than *T. bernacchii* and *T. pennellii* (**Figure 2**). When examined in relation to individual length, we found that *T. bernacchii* had much higher variability in CF than the

other three species. The high variability in CF in *T. bernacchii* appears to be driven by relatively high variability in standard length paired with extremely low variability in weight. Even with careful inspection, we found no evidence of experimental factors (i.e., measurer, experiment type, date) that could have artificially caused this finding. The high variability in CF in *T. bernacchii* may be related to a more generalist diet compared to the other three species, multiple energy allocation strategies, and/or higher intraspecific competition (Conrad *et al.*, 2011; Mittelbach *et al.*, 2014). It is also thought that variability in physiological status can drive divergent behavioral syndromes (Mittelbach *et al.*, 2014; Réale *et al*., 2010). The optimal behavioral strategy for an individual with limited energy reserves and/or metabolic capacity, for example, would be much different than the optimal behavioral strategy for a fish with abundant energy reserves (Réale *et al*., 2010). Such 'pace-of-life' syndromes need more exploration in Antarctic ecosystems, where, generally, the pace of life is incredibly slow due to such low temperatures. Even small variation in pace-of-life syndromes may have important implications for population and community dynamics in this ecosystem. Given that *T. bernacchii* are the most abundant and the most well-studied of these species, this finding certainly calls for further exploration.

We also found high intraspecific variability in RMR in *T. nicolai* and *P. borchgrevinki* when compared to *T. bernacchii* and *T. pennellii*. There are many drivers of intraspecific variation in RMR, including genotype, maternal effects, environmental conditions in early development, and behavioral syndromes (for a review, see Burton *et al.*, 2011). Because these fishes are all found in the same habitat and are closely related – and it is known that *T. nicolai* and *P. borchgrevinki* are more active at adult life

stages than *T. bernacchii* and *T. pennellii* – it is plausible that behavioral syndromes across a greater range of activity levels, or ecological “lifestyles”, are driving the heightened variation in RMR in *T. nicolai* and *P. borchgrevinki* (Killen *et al*., 2016). This is further supported by high variation in behavioral metrics in the NOT and NTT in *T. nicolai* and *P. borchgrevinki*. In the NOT, variation in the frequency of entering the center zone increased in response to the novel object in *T. nicolai* and *P. borchgrevinki*, suggesting divergent behavioral strategies in response to novelty, where certain individuals approach the novel object frequently and others completely avoid it.

We also found evidence of partitioning of two behavioral phenotypes within *T. nicolai* in the NTT, where approximately half of the individuals are highly exploratory (as measured by the frequency of entering the top zone) and the other half show low exploratory behavior (**Figure 5**). While the present study does not measure behavioral syndromes *per se*, as individuals were not measured more than once, the variability in exhibited behavior indicates the presence of distinct behavioral phenotypes. This apparent partitioning of exploratory-avoidant behaviors suggests that certain individuals exhibit higher exploratory tendencies, while others are more avoidant. Given the nearly even ratio of exploratory vs. avoidant behaviors, it is plausible that these behavioral differences may be due to sex, a phenomenon seen in many more well-studied fish species (Jackson *et al*., 2025; Pärssinen *et al*., 2021; Rajput *et al*., 2022; Wallace *et al*., 2020). Developing methods to sex individuals of these species – potentially via molecular markers or hormone assays – would greatly improve our understanding of these species; thus far, Antarctic fishes have demonstrated complex sex chromosome systems (Cocca *et al*., 2015; Ghigliotti *et al*., 2013; Ghigliotti *et al*., 2016; Morescalchi *et*

al., 1992). Further examining behavioral syndromes in these fishes, particularly in *T. nicolai*, is an important line of future research, as behavioral syndromes can shape survival, dispersal patterns, social structure, trophic interactions, predator-prey interactions, habitat use, foraging efficiency, and intraspecific competition (Conrad *et al*., 2011; Gosling, 2001; Réale *et al*., 2007; Sih *et al*., 2004b; Wilson *et al*., 1994). For example, individuals that are more exploratory may have a higher likelihood of encountering novel prey resources, while those that are more avoidant may be less prone to predation, leading to trade-offs that affect fitness and survival (Dingemanse and Réale, 2005; Smith and Blumstein, 2008). Critically, the social impacts of behavioral syndromes are species and context specific (Brown *et al.*, 2007a; Jones and Godin, 2010; Smith and Blumstein, 2008); while all of these dynamics would be expected to influence niche partitioning and performance in future ocean conditions, any adaptive (or maladaptive) consequences of varied behavioral syndromes are unknown.

Whether behavioral strategies are stable through ontogeny remains unclear, although some studies have demonstrated stability in single behaviors over time (Conrad *et al*., 2011). Given the genetic basis for personality differences, it is plausible that more exploratory – or avoidant – individuals continue this behavior into adulthood. This has significant ecological implications, such as habitat use and inter- and intraspecific interactions (Biro and Stamps, 2008; Conrad *et al*., 2011). Further studies on the fitness consequences of behavioral variation, its role in niche differentiation, and whether behavioral strategies persist across ontogenetic shifts – especially those associated with drastic habitat shifts – would provide valuable insight into how these

patterns influence population structure and species interactions in Antarctic fish communities.

Concluding remarks

Comparative approaches are integral to our efforts to predict how species, communities, and ecosystems may fare under the environmental challenges of a rapidly changing world. Antarctic ecosystems are facing many worsening, co-occurring stressors, including ocean warming, ocean acidification, habitat loss, pollutants and microplastics, fishing pressure, and invasions of more temperate species (Palmer *et al.*, 2022; Rintoul *et al*., 2018; Zheng et al., 2022). Habitats with high species richness and diversity, such as the near-shore fish communities on Antarctic shelves (Hanchet *et al*., 2013), are important for buffering the resilience of the larger ecosystem and are thought to be more resilient to ecosystem collapse (Loreau and de Mazancourt, 2013; MacArthur, 1955; Mazancourt *et al*., 2013; Tilman, 1996; Tilman, 1999). This is thought to be, at least in part, a lifeboat of functional redundancy; if one species suffers from a stressor (or many), it is thought that others could “swim in” and take over their role (Fetzer *et al*., 2015). This could lead to consequential shifts in ecological interactions and community structure, such as niche expansion or compression (for reviews, see Carscadden *et al*., 2020 and Sexton *et al*., 2017). However, we know that rare species can also play important functional roles in ecosystems (Dee *et al*., 2019). Because of the rapid rate of environmental change paired with the long life spans of these fishes, it is expected that these ecosystems may need to rely on functional redundancy rather than species adaptation. This study demonstrates that by characterizing subtle interspecific

differences, we can identify potential vulnerabilities that could drive intra- and interspecific performance in future oceans. We also emphasize the importance of situating physiological studies within the community ecology of the system. Our hope is that this work informs predictions on differential interspecific performance and helps identify priority species for future stressor exposure studies.

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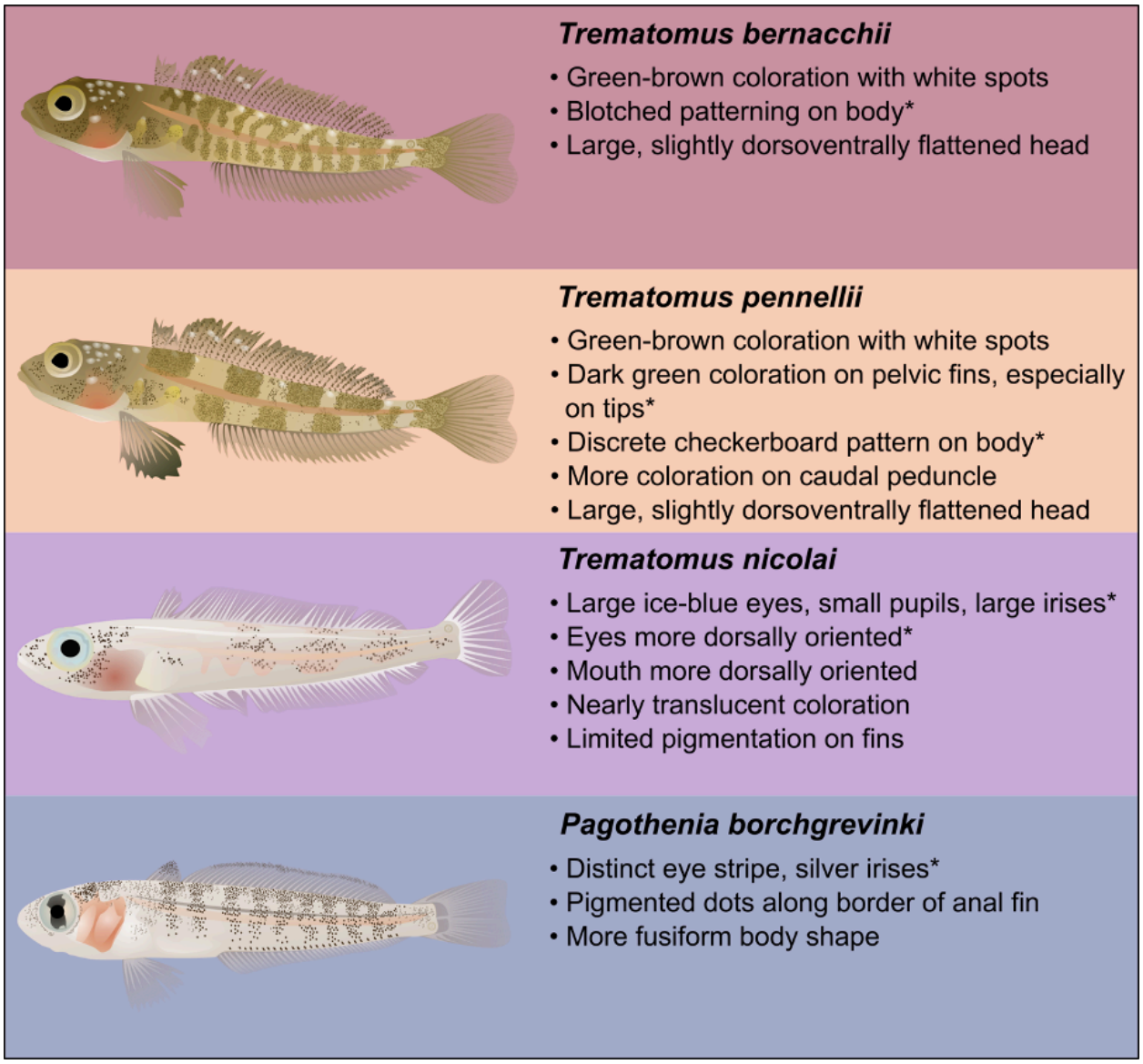


Figure 1. Identification guide for juvenile life stages of four abundant species in McMurdo Sound, Antarctica: T. bernacchii, T. pennellii, T. nicolai, and P. borchgrevinki. Illustrations are done by Dr. Jess McLaughlin. We include remarks of distinguishing features; the most defining features are starred.

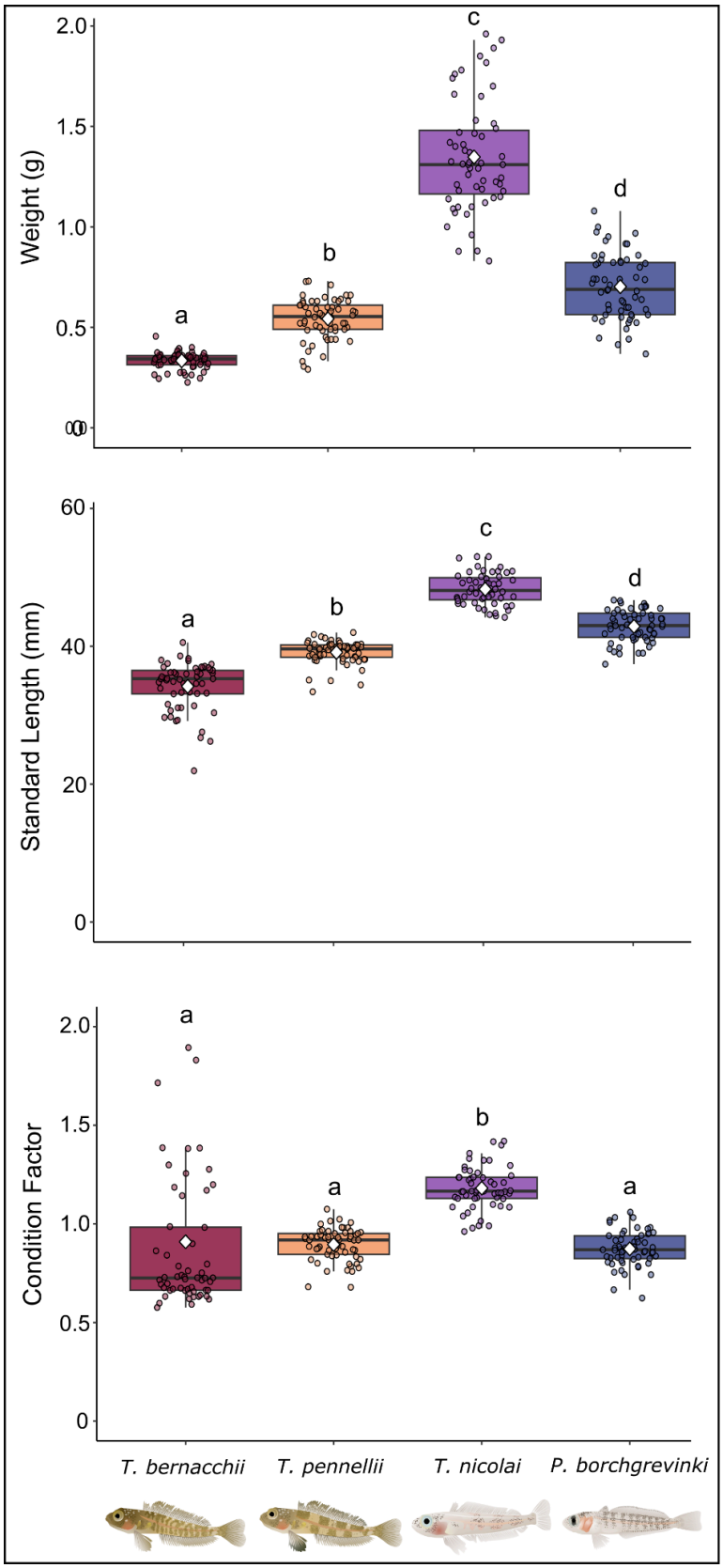


Figure 2. Morphometrics of juvenile *T. bernacchii* (maroon, n=57), *T. pennellii* (orange, n=61), *T. nicolai* (purple, n=55), and *P. borchgrevinki* (blue, n=57). (a): Fresh wet weight (g), (b): standard length (mm), (c): Fulton's condition factor. Data are presented as boxplots with centerline, box, and whiskers representing the median, interquartile range (IQR), and 1.5 times the IQR, respectively. Raw data are plotted overlaying the boxplots as points. Means are denoted by white diamonds. Letters indicate significant differences.

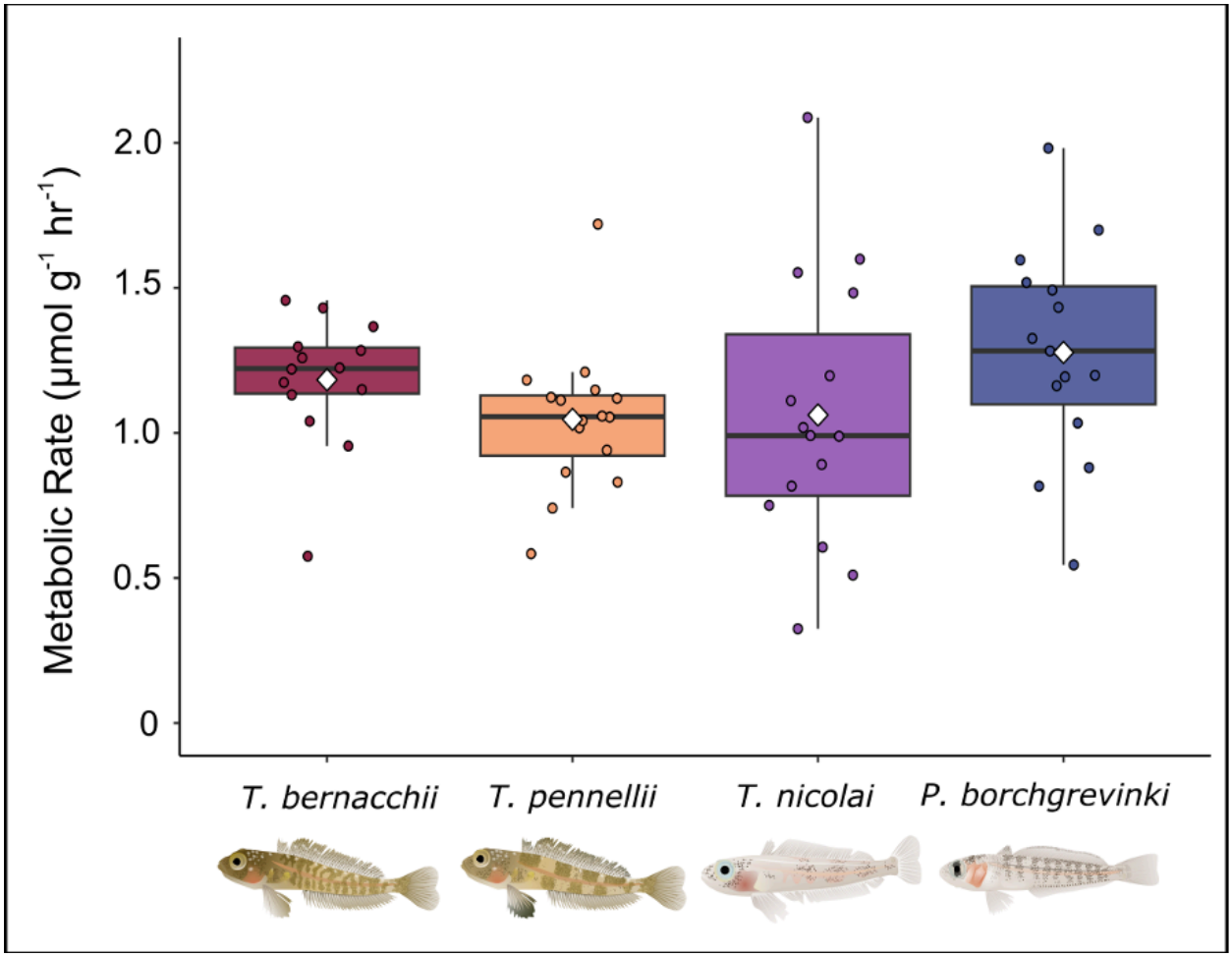


Figure 3. Mass-specific resting metabolic rate (mmol O_2 g^{-1} hr^{-1}) of juvenile *T. bernacchii* (maroon, n=14), *T. pennellii* (orange, n=15), *T. nicolai* (purple, n=15), and *P. borchgrevinki* (blue, n=15). Data are presented as boxplots with centerline, box, and whiskers representing the median, interquartile range (IQR), and 1.5 times the IQR, respectively. Raw data are plotted overlaying the boxplots as points. Means are denoted by white diamonds. There were no significant differences in metabolic rate among species.

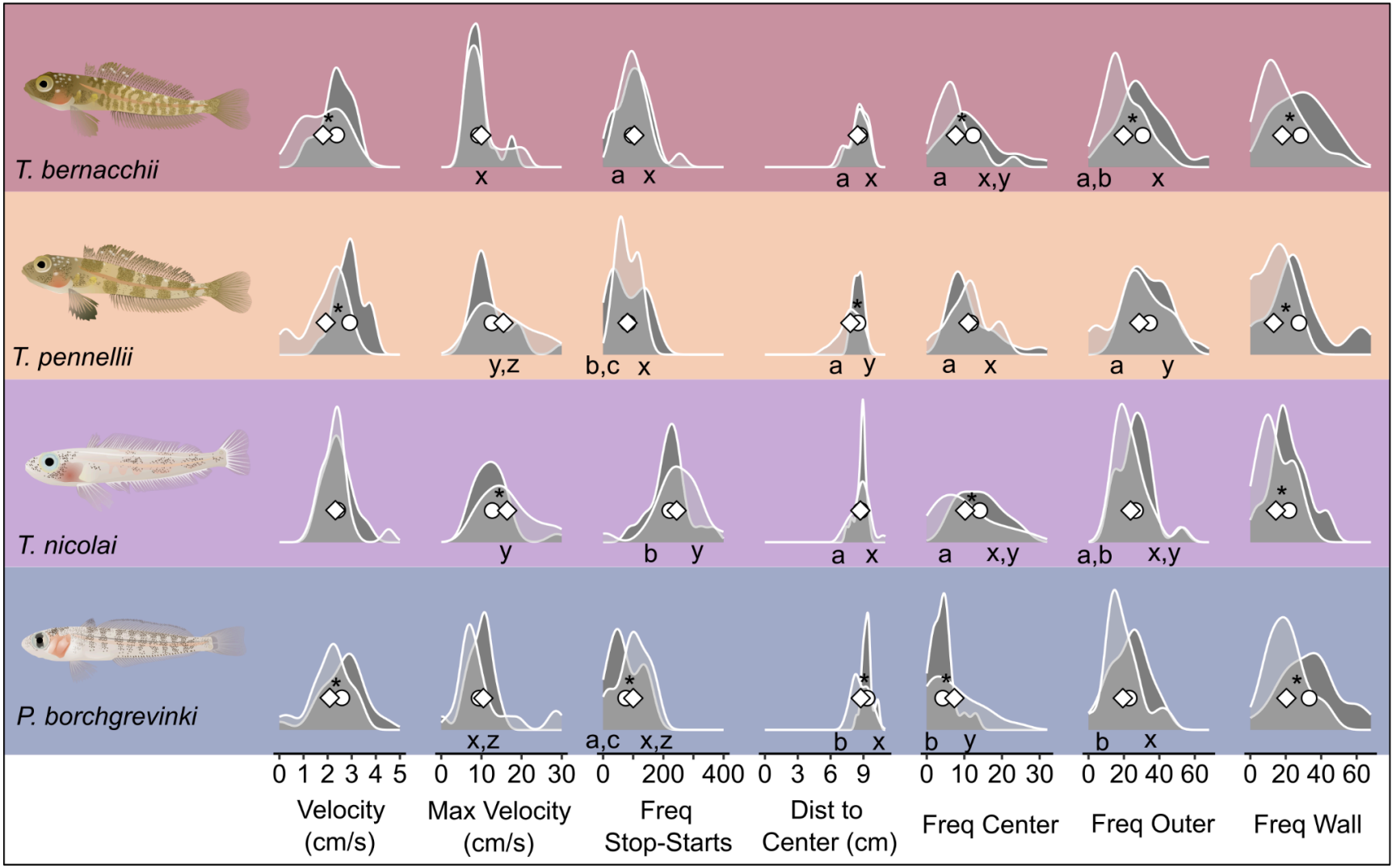

T. bernacchii
T. pennellii
T. nicolai
P. borchgrevinki
x
a x
a x
a x,y
a,b x
y,z
b,c x
a y
a x
a y
y
b y
a x
a x,y
a,b x,y
x,z
a,c x,z
b x
b y
b x
0 1 2 3 4 5
Velocity (cm/s)
0 10 20 30
Max Velocity (cm/s)
0 200 400
Freq Stop-Starts
0 3 6 9
Dist to Center (cm)
0 10 20 30
Freq Center
0 20 40 60
Freq Outer
0 20 40 60
Freq Wall

Figure 4.

Figure 4. NOT behavioral metrics of juvenile *T. bernacchii* (maroon, n=20), *T. pennellii* (orange, n=20), *T. nicolai* (purple, n=20), and *P. borchgrevinki* (blue, n=20). NOT metrics are presented along the x-axis and species are presented along the y-axis. NOT metrics include Velocity (cm/s), Maximum Velocity (cm/s), Frequency of Stop-Starts, Distance to Center Point (cm), Frequency Entering Center Zone, Frequency Entering Outer Zone, and Frequency Entering Wall Zone. Data are presented as density distributions, where shaded areas represent the estimated probability density of each metric, with dark grey and light grey indicating pre- and post- NO, respectively. White circles and diamonds denote means for pre- and post- NO, respectively. Letters indicate significant differences between species (a,b,c for pre- and x,y,z for post- NO, $p<0.05$) and stars signify significant differences within species after the novel object was added ($p<0.05$).

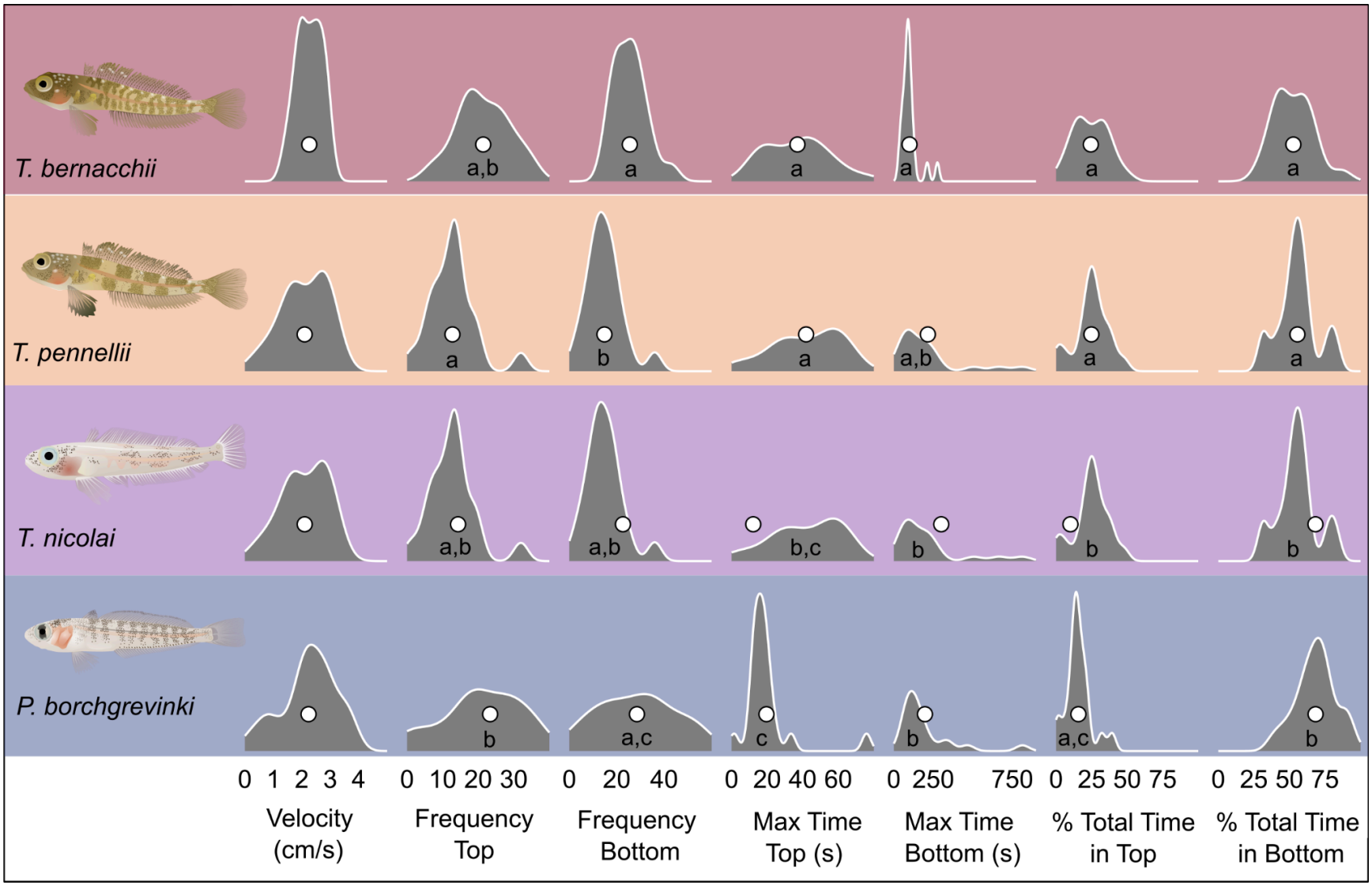

T. bernacchii
a,b
a
a
a
a
a
T. pennellii
a
b
a
a,b
a
a
T. nicolai
a,b
a,b
b,c
b
b
b
P. borchgrevinki
b
a,c
c
b
a,c
b
0 1 2 3 4
Velocity (cm/s)
0 10 20 30
Frequency Top
0 20 40
Frequency Bottom
0 20 40 60
Max Time Top (s)
0 250 750
Max Time Bottom (s)
0 25 50 75
% Total Time in Top
0 25 50 75
% Total Time in Bottom

Figure 5.

Figure 5. NTT behavioral metrics of juvenile *T. bernacchii* (maroon, n=20), *T. pennellii* (orange, n=20), *T. nicolai* (purple, n=18), and *P. borchgrevinki* (blue, n=20). NTT metrics are presented along the x-axis and species are presented along the y-axis. NTT metrics include Velocity (cm/s), Frequency Entering Top Zone, Frequency Entering Bottom Zone, Maximum Time in Top Zone (s), Maximum Time in Bottom Zone (s), Percent Time in Top Zone, and Percent Time in Bottom Zone. Data are presented as density distributions, where shaded areas represent the estimated probability density of each metric. White points denote means and letters indicate significant differences between species.

Appendix

S. Table 1. Primer sequences and PCR protocols for the three genes used to barcode *T. bernacchii*, *T. pennellii*, *T. nicolai*, and *P. borchgrevinki*: cytochrome c oxidase subunit I (COI), NADH dehydrogenase subunit 2 (ND2), and cytochrome b (Cyt b).

Gene	Primer Sequence	PCR Protocol	Reference
COI	Forward1: 5'- TGT AAA ACG ACG GCC AGT CAA CCA ACC ACA AAG ACA TTG GCA C-3'	*Initial denaturation:* 94ºC 2 min *Amplification:* 94ºC 30 s 54ºC 40 s 72ºC 1 min 35 cycles *Final extension:* 72ºC 10 min 4ºC indefinite hold	Ivanova *et al.* 2007 (VF2_t1, FishF2t1, FishR2_t1, FR1d_t1)
	Forward2: 5'- TGT AAA ACG ACG GCC AGT CGA CTA ATC ATA AAG ATA TCG GCA C-3'		
	Reverse1: 5'- CAG GAA ACA GCT ATG ACA CTT CAG GGT GAC CGA AGA ATC AGA A-3'		
	Reverse2: 5'- CAG GAA ACA GCT ATG ACA CCT CAG GGT GTC CGA ARA AYC ARA A-3'		
ND2	Forward: 5'- CTA CCT GAA GAG ATC AAA AC-3'	*Initial denaturation:* 94ºC 3 min *Amplification:* 94ºC 30 s 55ºC 1 min 72ºC 90 s 35 cycles *Final extension:* 72ºC 5 min 4ºC indefinite hold	Kocher *et al.* 1995
	Reverse: 5'- CGC GTT TAG CTG TTA ACT AA-3'		
Cyt b	Forward: 5'- CGC GAC TGT AAT TAC TAA CCT C-3'	*Initial denaturation:* 94ºC 3 min *Amplification:* 94ºC 1 min 56ºC 1 min 72ºC 1 min 35 cycles *Final extension:* 72ºC 8 min 4ºC indefinite hold	NA; custom for this study
	Reverse: 5'- CGA TTT GGC CGA TAA TGA TGT A-3'		

S. Table 2. Summary of biologically informed backwards model selection used for all models. Where multiple models were tested, the best fitting model is in bold text.

MORPHOMETRICS									
Model	Model Structure	Model d.f.	Dropped Test Parameter	AIC	BIC	L Ratio	log-likelihood	Chisq	p-value
Weight (g)									
Final	lme(Weight ~ Species + 1|Expt, weights = Species)	9		-303.54	-272.76		160.77		
Length (mm)									
Final	lme(Length ~ Species + 1|Expt, weights = Species)	9		1059.02	1089.81		-520.51		
Condition Factor									
Final	lme(Condition ~ Species + 1|Expt, weights = Species)	9		-236.69	-205.90		127.34		
METABOLIC RATE									
Model	Model Structure	Model d.f.	Dropped Test Parameter	AIC	BIC	L Ratio	log-likelihood	Chisq	p-value
Mass Specific Metabolic Rate (μmol g-1 hr-1)									
Ref	lme(MO2 ~ Species + Weight + Length + 1|Chamber, weights = Species)	11		121.52	144.55		-49.76		
1	lme(MO2 ~ Species + Weight + 1|Chamber, weights = Species)	10	Length	125.19	146.13	5.68	-52.60		0.02
2/Final	**lme(MO2 ~ Species + Length + 1|Chamber, weights = Species)**	10	Weight	121.90	142.85	2.39	-50.95		0.12
NOVEL OBJECT									
Model	Model Structure	Model d.f.	Dropped Test Parameter	AIC	BIC	L Ratio	log-likelihood	Chisq	p-value
Mean Velocity (cm/s)									
Ref/Final	**lme(Velocity ~ Species*NO + Weight + Length + 1|ID + 1|Bucket, weights = Species)**	16		300.83	349.63		-134.42		
1	lme(Velocity ~ Species*NO + Weight + 1|ID + 1|Bucket,	15	Length	302.61	348.36	3.78	-136.31		0.05

	weights = Species)								
2	lme(Velocity ~ Species*NO + Length + 1|ID + 1|Bucket, weights = Species)	15	Weight	302.51	348.26	3.68	-136.26		0.06
Maximum Velocity (cm/s)									
Ref	glmer(Max Velocity ~ Species*NO + Weight + Length + 1|ID + 1|Bucket, family = gamma)	13		872.10	911.75		-423.05		
1	glmer(Max Velocity ~ Species*NO + Weight + 1|ID + 1|Bucket, family = gamma)	12	Length	872.76	909.36		-424.38	2.67	0.10
2	glmer(Max Velocity ~ Species*NO + Length + 1|ID + 1|Bucket, family = gamma)	12	Weight	872.34	908.94		-424.17	2.24	0.14
Final	**glmer(Max Velocity ~ Species*NO + 1|ID + 1|Bucket, family = gamma)**	11		870.78	904.32		-424.39		
Number of Start-Stop's									
Final	glmmTMB(Start-Stop ~ Species*NO + 1|ID + 1|Bucket, family=nbinom2)	11		1798.30	1832.13		-888.15		
Mean Distance to Center Point (cm)									
Final	lme(Distance ~ Species*NO + 1|ID + 1|Bucket, weights = Species)	14		328.11	370.81		-150.05		
Frequency Entering Center Zone									
Final	glmmTMB(Center Frequency ~ Species*NO + 1|ID + 1|Bucket, family=nbinom2)	11		1007.67	1041.49		-492.83		
Frequency Entering Outer Zone									
Final	glmmTMB(Outer Frequency ~ Species*NO + 1|ID + 1|Bucket, family=nbinom2)	11		1249.88	1283.71		-613.94		
Frequency Entering Wall Zone									
Final	glmmTMB(Wall Frequency ~ Species*NO + 1|ID + 1|Bucket, family=nbinom2)	11		1279.52	1313.34		-628.76		
NOVEL TANK									
Model	Model Structure	Model d.f.	Dropped Test Parameter	AIC	BIC	L Ratio	log-likelihood	Chisq	p-value
Mean Velocity (cm/s)									
Ref	lme(Velocity ~ Species + Weight + Length + 1|Tank, weights = Species)	11		162.25	187.14		-70.12		
1	lme(Velocity ~ Species + Weight + 1|Tank, weights = Species)	10	Length	160.25	182.88	0.004	-70.13		0.95

2	lme(Velocity ~ Species + Length + 1|Tank, weights = Species)	10	Weight	161.29	183.92	1.05	-70.65		0.31
Final	lme(Velocity ~ Species + 1|Tank, weights = Species)	9		159.41	179.77		-70.71		
Frequency Entering Top Zone									
Final	glmmTMB(Top Frequency ~ Species + 1|Tank)	6		530.14	543.71		-259.07		
Frequency Entering Bottom Zone									
Final	glmmTMB(Bottom Frequency ~ Species + 1|Tank)	6		545.35	558.92		-266.67		
Max Time in Top Zone									
Final	lme(Max Time ~ Species + 1|Tank, weights=Species)	9		586.32	606.68		-284.16		
Max Time in Bottom Zone									
Final	lme(log Max Time ~ Species + 1|Tank, weights=Species)	9		149.80	170.16		-65.90		
Percent of Time in Top Zone									
Final	glmmTMB(Percent Time ~ Species + 1|Tank)	6		-115.83	-102.26		63.92		
Percent of Time in Bottom Zone									
Final	glmmTMB(Percent Time ~ Species + 1|Tank)	6		-76.11	-62.54		44.06		

S. Table 3. Model summaries of all final models

MORPHOMETRICS						
Weight (g)						
Model: lme(Weight ~ Species + 1|Expt, weights = Species) *Marginal* R^2 = 0.654, *conditional* R^2 = 0.656						
Fixed Effect	Variance parameter (σ)	Estimate	s.e.	df	t-value	p-value
Intercept		0.33	0.01	224	26.21	<0.0001
Species - *T. bernacchii*	0.15					
Species - *T. pennellii*	0.36	0.21	0.01	224	15.32	<0.0001
Species - *T. nicolai*	1.00	1.02	0.04	224	27.39	<0.0001
Species - *P. borchgrevinki*	0.60	0.37	0.02	224	16.59	<0.0001
Random Effect						
Expt variance		0.02				
Residual variance		0.27				
Standard Length (mm)						
Model: lme(Length ~ Species + 1|Expt, weights = Species) *Marginal* R^2 = 0.835, *conditional* R^2 = 0.836						
Fixed Effect	Variance parameter (σ)	Estimate	s.e.	df	t-value	p-value
Intercept		34.18	0.48	224	70.67	<0.0001
Species - *T. bernacchii*	1.55					
Species - *T. pennellii*	0.75	4.92	0.51	224	9.59	<0.0001
Species - *T. nicolai*	1.00	14.11	0.56	224	25.34	<0.0001
Species - *P. borchgrevinki*	0.98	8.71	0.55	224	15.80	<0.0001
Random Effect						
Expt variance		0.23				
Residual variance		2.27				
Condition Factor						
Model: lme(Condition ~ Species + 1|Expt, weights = Species) *Marginal* R^2 = 0.586, *conditional* R^2 = 0.611						
Fixed Effect	Variance parameter (σ)	Estimate	s.e.	df	t-value	p-value
Intercept		0.91	0.06	224	15.19	<0.0001
Species - *T. bernacchii*	4.29					
Species - *T. pennellii*	0.80	-0.01	0.06	224	-0.23	0.82
Species - *T. nicolai*	1.00	0.27	0.06	224	4.62	<0.0001
Species - *P. borchgrevinki*	0.85	-0.04	0.06	224	-0.61	0.54
Random Effect						
Expt variance		0.03				
Residual variance		0.10				

METABOLIC RATE						
Mass Specific Metabolic Rate (μmol g-1 hr-1)						
Model: lme(MO2 ~ Species + Length + 1|Chamber, weights = Species) *Marginal* R^2 = 0.143, *conditional* R^2 = 0.372						
Fixed Effect	Variance parameter (σ)	Estimate	s.e.	df	t-value	p-value
Intercept		5.88	1.84	39	3.20	0.003
Species - *T. bernacchii*	0.69					
Species - *T. pennellii*	0.99	0.14	0.30	16	0.47	0.64
Species - *T. nicolai*	1.89	1.04	0.70	16	1.49	0.16
Species - *P. borchgrevinki*	1.00	0.76	0.31	39	2.49	0.02
Length		0.10	0.05	39	-1.95	0.06
Random Effect						
Chamber variance		0.30				
Residual variance		0.49				
NOVEL OBJECT						
Mean Velocity (cm/s)						
Model: lme(Velocity ~ Species*NO + Weight + Length + 1|ID + 1|Bucket, weights = Species) *Marginal* R^2 = 0.200, *conditional* R^2 = 0.884						
Fixed Effect	Variance parameter (σ)	Estimate	s.e.	df	t-value	p-value
Intercept		6.77	3.44	73	1.97	0.053
Species - *T. bernacchii*	1.56					
Species - *T. pennellii*	2.15	0.64	0.31	73	2.04	0.04
Species - *T. nicolai*	1.06	0.23	0.61	73	0.38	0.71
Species - *P. borchgrevinki*	1.00	0.55	0.49	73	1.12	0.27
NO - Post		-0.57	0.13	69	-4.26	0.0001
Weight		1.47	1.04	73	1.41	0.16
SL		-0.13	0.10	73	-1.30	0.20
T. pennellii x NO Post		-0.38	0.24	69	-1.58	0.12
T. nicolai x NO Post		0.45	0.16	69	2.76	0.01
P. borchgrevinki x NO Post		0.07	0.16	69	0.42	0.68
Random Effect						
ID variance		<0.0001				
Bucket variance		0.66				
Residual variance		0.27				
Maximum Velocity (cm/s)						
Model: glmer(Max Velocity ~ Species*NO + 1|ID + 1|Bucket, family = Gamma) *Marginal* R^2 < 0.01, *conditional* R^2 < 0.01						
Fixed Effect		Estimate	s.e.		t-value	p-value

Intercept		0.117	0.01		10.81	<0.0001
Species - *T. pennellii*		-0.027	0.01		-1.85	0.06
Species - *T. nicolai*		-0.027	0.01		-1.86	0.06
Species - *P. borchgrevinki*		0.002	0.01		0.12	0.90
NO - Post		-0.007	0.01		-0.72	0.47
T. pennellii x NO Post		-0.006	0.01		-0.51	0.61
T. nicolai x NO Post		-0.009	0.01		-0.73	0.47
P. borchgrevinki x NO Post		-0.003	0.01		-0.18	0.85
Random Effect						
ID variance		0.001				
Bucket variance		<0.0001				
Residual variance		0.120				
Adjusted ICC		0.004				
Number of Stop/Starts						
Model: glmmTMB(Start Stops ~ Species*NO + 1|ID + 1|Bucket, family=nbinom2) *Marginal* R^2 = 0.315, *conditional* R^2 = 0.730						
Fixed Effect		Estimate	s.e.		z-value	p-value
Intercept		4.44	0.17		26.40	<0.0001
Species - *T. pennellii*		-0.22	0.22		-0.99	0.32
Species - *T. nicolai*		0.93	0.22		4.26	<0.0001
Species - *P. borchgrevinki*		-0.37	0.22		-1.67	0.09
NO - Post		0.11	0.14		0.79	0.43
T. pennellii x NO Post		-0.01	0.20		-0.07	0.94
T. nicolai x NO Post		-0.05	0.19		-0.27	0.79
P. borchgrevinki x NO Post		0.24	0.20		1.21	0.22
Random Effect						
ID variance		0.29				
Bucket variance		0.02				
Mean Distance to Center Point (cm)						
Model: lme(Distance ~ Species*NO + 1|ID + 1|Bucket, weights = Species) *Marginal* R^2 = 0.253, *conditional* R^2 = 0.757						
Fixed Effect	Variance parameter (σ)	Estimate	s.e.	df	t-value	p-value
Intercept		8.67	0.15	76	58.88	<0.0001
Species - *T. bernacchii*	0.87					
Species - *T. pennellii*	1.76	-0.10	0.24	76	-0.42	0.67
Species - *T. nicolai*	1.23	0.08	0.22	76	0.36	0.72
Species - *P. borchgrevinki*	1.00	0.66	0.21	76	3.11	0.003
NO - Post		-0.21	0.11	70	-1.91	0.06
T. pennellii x NO Post		-0.57	0.26	70	-2.24	0.03

T. nicolai x NO Post		0.17	0.19	70	0.89	0.38
P. borchgrevinki x NO Post		-0.38	0.16	70	-2.35	0.02
Random Effect						
ID variance		<0.001				
Bucket variance		0.56				
Residual variance		0.39				
Frequency Entering Center Zone						
Model: glmmTMB(Center Frequency ~ Species*NO + 1|ID + 1|Bucket, family=nbinom2) *Marginal* R^2 = 0.245, *conditional* R^2 = 0.494						
Fixed Effect		Estimate	s.e.		z-value	p-value
Intercept		2.43	0.14		17.05	<0.0001
Species - *T. pennellii*		-0.003	0.20		-0.02	0.99
Species - *T. nicolai*		0.17	0.20		0.88	0.38
Species - *P. borchgrevinki*		-1.06	0.22		-4.83	<0.0001
NO - Post		-0.48	0.17		-2.87	0.004
T. pennellii x NO Post		0.43	0.23		1.84	0.07
T. nicolai x NO Post		0.09	0.23		0.39	0.70
P. borchgrevinki x NO Post		0.98	0.25		3.85	0.0001
Random Effect						
ID variance		0.15				
Bucket variance		<0.0001				
Adjusted ICC		0.39				
Frequency Entering Outer Zone						
Model: glmmTMB(Outer Frequency ~ Species*NO + 1|ID + 1|Bucket, family=nbinom2) *Marginal* R^2 = 0.144, *conditional* R^2 = 0.488						
Effect		Estimate	s.e.		z-value	p-value
Intercept		3.35	0.11		30.59	<0.0001
Species - *T. pennellii*		0.17	0.15		1.09	0.28
Species - *T. nicolai*		-0.11	0.16		-0.71	0.48
Species - *P. borchgrevinki*		-0.29	0.16		-1.85	0.06
NO - Post		-0.44	0.12		-3.63	0.0003
T. pennellii x NO Post		0.22	0.17		1.31	0.19
T. nicolai x NO Post		0.32	0.17		1.87	0.06
P. borchgrevinki x NO Post		0.27	0.17		1.53	0.13
Random Effect						
ID variance		0.10				
Bucket variance		<0.0001				
Adjusted ICC		0.44				
Frequency Entering Wall Zone						

Model: glmmTMB(Wall Frequency ~ Species*NO + 1|ID + 1|Bucket, family=nbinom2) *Marginal R^2* = 0.191, *conditional R^2* = 0.590						
Effect		Estimate	s.e.		z-value	p-value
Intercept		3.27	0.16		20.68	
Species - *T. pennellii*		-0.04	0.22		-0.20	0.84
Species - *T. nicolai*		-0.26	0.22		-1.18	0.24
Species - *P. borchgrevinki*		0.16	0.22		0.74	0.46
NO - Post		-0.52	0.16		-3.17	0.002
T. pennellii x NO Post		-0.24	0.24		-0.99	0.32
T. nicolai x NO Post		0.07	0.23		0.28	0.78
P. borchgrevinki x NO Post		0.02	0.23		0.11	0.92
Random Effect						
ID variance		0.22				
Bucket variance		0.004				
Adjusted ICC		0.49				
NOVEL TANK						
Mean Velocity						
Model: lme(Mean Velocity ~ Species + 1|Tank, weights = Species) *Marginal R^2*=0.028, *conditional R^2* = 0.028						
Fixed Effect	Variance parameter (σ)	Estimate	s.e.	df	t-value	p-value
Intercept		2.26	0.10	72	22.86	<0.0001
Species - *T. bernacchii*	1.00					
Species - *T. pennellii*	1.83	-0.16	0.21	72	-0.79	0.43
Species - *T. nicolai*	2.27	-0.16	0.26	72	-0.63	0.53
Species - *P. borchgrevinki*	2.18	-0.03	0.24	72	-0.11	0.92
Random Effect						
Tank variance		<0.0001				
Residual variance		0.44				
Frequency Entering Top Zone						
Model: glmmTMB(Top Frequency ~ Species + 1|Tank) *Marginal R^2* = 0.129, *conditional R^2* = 0.129						
Fixed Effect		Estimate	s.e.		z-value	p-value
Intercept		3.06	0.17		18.14	<0.0001
Species - *T. pennellii*		-0.52	0.24		-2.14	0.03
Species - *T. nicolai*		-0.40	0.25		-1.62	0.11
Species - *P. borchgrevinki*		0.09	0.24		0.37	0.71
Random Effect						
Tank variance		<0.0001				
Frequency Entering Bottom Zone						
Model: glmmTMB(Bottom Frequency ~ Species + (1|Tank) *Marginal R^2* = 0.110, *conditional R^2* = 0.110						

Fixed Effect		Estimate	s.e.		z-value	p-value
Intercept		3.24	0.14		22.50	<0.0001
Species - *T. pennellii*		-0.54	0.21		-2.63	0.009
Species - *T. nicolai*		-0.12	0.21		-0.57	0.57
Species - *P. borchgrevinki*		0.11	0.20		0.55	0.58
Random Effect						
Tank variance		<0.0001				
Max Time in Top Zone						
Model: lme(Max Time ~ Species + 1|Tank, weights = Species) *Marginal* R^2 = 0.326, *conditional* R^2 = 0.326						
Fixed Effect	Variance parameter (σ)	Estimate	s.e.	df	t-value	p-value
Intercept		37.05	3.92	72	9.46	<0.0001
Species - *T. bernacchii*	1.00					
Species - *T. pennellii*	1.08	4.86	5.78	72	0.84	0.40
Species - *T. nicolai*	0.49	-24.88	4.42	72	-5.64	<0.0001
Species - *P. borchgrevinki*	0.84	-17.44	5.11	72	-3.42	0.001
Random Effect						
Tank variance		0.001				
Residual variance		17.51				
Max Time in Bottom Zone						
Model: lme(Max Time ~ Species + 1|Tank, weights = Species) *Marginal* R^2 = 0.260, *conditional* R^2 = 0.272						
Fixed Effect	Variance parameter (σ)	Estimate	s.e.	df	t-value	p-value
Intercept		4.49	0.11	72	40.53	<0.0001
Species - *T. bernacchii*	1.00					
Species - *T. pennellii*	1.84	0.50	0.22	72	2.27	0.03
Species - *T. nicolai*	2.18	0.77	0.26	72	2.90	0.005
Species - *P. borchgrevinki*	1.45	0.54	0.19	72	2.89	0.005
Random Effect						
Tank variance		0.06				
Residual variance		0.47				
Percent Time in Top Zone						
Model: glmmTMB(Percent Time ~ Species + 1|Tank) *Marginal* R^2 = 0.548, *conditional* R^2 = 0.553						
Fixed Effect		Estimate	s.e.		z-value	p-value
Intercept		-1.06	0.18		-5.84	<0.0001
Species - *T. pennellii*		-0.08	0.26		-0.31	0.75
Species - *T. nicolai*		-1.88	0.33		-5.76	<0.0001
Species - *P. borchgrevinki*		-0.53	0.26		-2.04	0.04
Random Effect						

Tank variance		0.005				
Percent Time in Bottom Zone						
Model: glmmTMB(Percent Time ~ Species + 1|Tank) *Marginal* R^2 = 0.630, *conditional* R^2 = 0.630						
Fixed Effect		Estimate	s.e.		t-value	p-value
Intercept		0.12	0.14		0.88	0.38
Species - *T. pennellii*		0.11	0.19		0.55	0.58
Species - *T. nicolai*		0.69	0.21		3.35	0.001
Species - *P. borchgrevinki*		0.65	0.20		3.27	0.001
Random Effect						
Tank variance		<0.0001				

S. Table 4. Adjusted contrasts from post-hoc significance testing for all models. Reported p-values were adjusted using the Benjamini-Hochberg method.

contrasts	estimate	s.e.	df	t- ratio or z-ratio	adjusted p-value
MORPHOMETRICS					
Weight (g)					
T. bernacchii - T. pennellii	-0.209	0.031	226	-6.763	**0.0001**
T. bernacchii - T. nicolai	-1.014	0.032	226	-32.042	**0.0001**
T. bernacchii - P. borchgrevinki	-0.368	0.031	226	-11.751	**0.0001**
T. pennellii - T. nicolai	-0.805	0.031	226	-25.870	**0.0001**
T. pennellii - P. borchgrevinki	-0.160	0.031	226	-5.185	**0.0001**
T. nicolai - P. borchgrevinki	0.645	0.032	226	20.396	**0.0001**
Standard Length (mm)					
T. bernacchii - T. pennellii	-4.920	0.514	224	-9.586	**0.0001**
T. bernacchii - T. nicolai	-14.110	0.557	224	-25.338	**0.0001**
T. bernacchii - P. borchgrevinki	-8.710	0.551	224	-15.802	**0.0001**
T. pennellii - T. nicolai	-9.180	0.375	224	-24.478	**0.0001**
T. pennellii - P. borchgrevinki	-3.790	0.367	224	-10.319	**0.0001**
T. nicolai - P. borchgrevinki	5.400	0.425	224	12.697	**0.0001**
Condition Factor					
T. bernacchii - T. pennellii	0.014	0.059	224	0.234	0.815
T. bernacchii - T. nicolai	-0.274	0.059	224	-4.615	**0.0001**
T. bernacchii - P. borchgrevinki	0.036	0.059	224	0.609	0.575
T. pennellii - T. nicolai	-0.288	0.017	224	-16.700	**0.0001**
T. pennellii - P. borchgrevinki	0.022	0.015	224	1.433	0.172
T. nicolai - P. borchgrevinki	0.310	0.018	224	17.360	**0.0001**
METABOLIC RATE					
Mass Specific Metabolic Rate (μmol g^{-1} hr^{-1})					
T. bernacchii - T. pennellii	-0.143	0.301	16	-0.475	0.964
T. bernacchii - T. nicolai	-1.038	0.697	16	-1.489	0.466
T. bernacchii - P. borchgrevinki	-0.761	0.306	39	-2.492	0.077
T. pennellii - T. nicolai	-0.895	0.554	16	-1.617	0.397
T. pennellii - P. borchgrevinki	-0.619	0.265	16	-2.333	0.132
T. nicolai - P. borchgrevinki	0.277	0.483	16	0.572	0.939
NOVEL OBJECT					
Mean Velocity (cm/s)					
T. bernacchii: pre - post NO	0.570	0.134	69	4.257	**0.001**

T. pennellii: pre - post NO	0.954	0.202	69	4.713	**0.001**
T. nicolai: pre - post NO	0.119	0.094	69	1.268	0.390
P. borchgrevinki: pre - post NO	0.503	0.086	69	5.870	**0.001**
Pre NO: *T. bernacchii - T. pennellii*	-0.642	0.314	73	-2.043	0.123
Pre NO: *T. bernacchii - T. nicolai*	-0.232	0.614	73	-0.377	0.800
Pre NO: *T. bernacchii - P. borchgrevinki*	-0.552	0.494	73	-1.118	0.448
Pre NO: *T. pennellii - T. nicolai*	0.411	0.467	73	0.880	0.528
Pre NO: *T. pennellii - P. borchgrevinki*	0.090	0.359	73	0.251	0.873
Pre NO: *T. nicolai - P. borchgrevinki*	-0.321	0.332	73	-0.967	0.497
Post NO: *T. bernacchii - T. pennellii*	-0.258	0.314	69	-0.822	0.552
Post NO: *T. bernacchii - T. nicolai*	-0.683	0.614	69	-1.112	0.448
Post NO: *T. bernacchii - P. borchgrevinki*	-0.619	0.494	69	-1.253	0.395
Post NO: *T. pennellii - T. nicolai*	-0.424	0.467	69	-0.909	0.522
Post NO: *T. pennellii - P. borchgrevinki*	-0.360	0.359	69	-1.005	0.486
Post NO: *T. nicolai - P. borchgrevinki*	0.064	0.332	69	0.192	0.896
Maximum Velocity (cm/s)					
T. bernacchii: pre - post NO	0.007	0.010	Inf	0.715	0.594
T. pennellii: pre - post NO	0.014	0.007	Inf	1.931	0.135
T. nicolai: pre - post NO	0.016	0.007	Inf	2.377	**0.051**
P. borchgrevinki: pre - post NO	0.010	0.010	Inf	1.009	0.486
Pre NO: *T. bernacchii - T. pennellii*	0.027	0.015	Inf	1.850	0.143
Pre NO: *T. bernacchii - T. nicolai*	0.027	0.015	Inf	1.860	0.143
Pre NO: *T. bernacchii - P. borchgrevinki*	-0.002	0.015	Inf	-0.122	0.928
Pre NO: *T. pennellii - T. nicolai*	0.000	0.014	Inf	0.015	0.988
Pre NO: *T. pennellii - P. borchgrevinki*	-0.029	0.015	Inf	-1.975	0.125
Pre NO: *T. nicolai - P. borchgrevinki*	-0.029	0.015	Inf	-1.984	0.125
Post NO: *T. bernacchii - T. pennellii*	0.033	0.014	Inf	2.391	**0.051**
Post NO: *T. bernacchii - T. nicolai*	0.036	0.014	Inf	2.602	**0.035**
Post NO: *T. bernacchii - P. borchgrevinki*	0.001	0.014	Inf	0.052	0.975
Post NO: *T. pennellii - T. nicolai*	0.003	0.013	Inf	0.217	0.892
Post NO: *T. pennellii - P. borchgrevinki*	-0.032	0.014	Inf	-2.350	0.056
Post NO: *T. nicolai - P. borchgrevinki*	-0.035	0.014	Inf	-2.562	**0.037**
Number of Start/Stop's					
T. bernacchii: pre - post NO	-0.110	0.138	Inf	-0.791	0.565
T. pennellii: pre - post NO	-0.095	0.143	Inf	-0.666	0.615
T. nicolai: pre - post NO	-0.057	0.135	Inf	-0.424	0.785
P. borchgrevinki: pre - post NO	-0.349	0.141	Inf	-2.479	**0.046**
Pre NO: *T. bernacchii - T. pennellii*	0.219	0.220	Inf	0.993	0.486
Pre NO: *T. bernacchii - T. nicolai*	-0.929	0.218	Inf	-4.257	**0.001**

Pre NO: *T. bernacchii - P. borchgrevinki*	0.369	0.221	Inf	1.671	0.201
Pre NO: *T. pennellii - T. nicolai*	-1.148	0.219	Inf	-5.235	**0.001**
Pre NO: *T. pennellii - P. borchgrevinki*	0.150	0.221	Inf	0.678	0.613
Pre NO: *T. nicolai - P. borchgrevinki*	1.298	0.220	Inf	5.907	**0.001**
Post NO: *T. bernacchii - T. pennellii*	0.233	0.219	Inf	1.063	0.461
Post NO: *T. bernacchii - T. nicolai*	-0.877	0.218	Inf	-4.026	**0.001**
Post NO: *T. bernacchii - P. borchgrevinki*	0.129	0.219	Inf	0.589	0.670
Post NO: *T. pennellii - T. nicolai*	-1.110	0.218	Inf	-5.088	**0.001**
Post NO: *T. pennellii - P. borchgrevinki*	-0.104	0.220	Inf	-0.471	0.760
Post NO: *T. nicolai - P. borchgrevinki*	1.006	0.219	Inf	4.600	**0.001**
Mean Distance to Center Point (cm)					
T. bernacchii: pre - post NO	0.206	0.108	70	1.912	0.143
T. pennellii: pre - post NO	0.779	0.232	70	3.351	**0.006**
T. nicolai: pre - post NO	0.040	0.152	70	0.262	0.872
P. borchgrevinki: pre - post NO	0.591	0.124	70	4.770	**0.001**
Pre NO: *T. bernacchii - T. pennellii*	0.104	0.245	76	0.424	0.785
Pre NO: *T. bernacchii - T. nicolai*	-0.079	0.222	76	-0.358	0.808
Pre NO: *T. bernacchii - P. borchgrevinki*	-0.662	0.213	76	-3.110	**0.015**
Pre NO: *T. pennellii - T. nicolai*	-0.183	0.257	76	-0.714	0.594
Pre NO: *T. pennellii - P. borchgrevinki*	-0.766	0.249	76	-3.078	**0.015**
Pre NO: *T. nicolai - P. borchgrevinki*	-0.582	0.226	76	-2.576	**0.043**
Post NO: *T. bernacchii - T. pennellii*	0.677	0.245	70	2.763	**0.030**
Post NO: *T. bernacchii - T. nicolai*	-0.245	0.222	70	-1.105	0.448
Post NO: *T. bernacchii - P. borchgrevinki*	-0.277	0.213	70	-1.301	0.382
Post NO: *T. pennellii - T. nicolai*	-0.922	0.257	70	-3.593	**0.006**
Post NO: *T. pennellii - P. borchgrevinki*	-0.954	0.249	70	-3.834	**0.002**
Post NO: *T. nicolai - P. borchgrevinki*	-0.032	0.226	70	-0.141	0.922
Frequency Entering Center Zone					
T. bernacchii: pre - post NO	0.477	0.166	Inf	2.868	**0.019**
T. pennellii: pre - post NO	0.0511	0.161	Inf	0.317	0.833
T. nicolai: pre - post NO	0.3879	0.16	Inf	2.421	**0.051**
P. borchgrevinki: pre - post NO	-0.4997	0.192	Inf	-2.607	**0.035**
Pre NO: *T. bernacchii - T. pennellii*	0.0034	0.2	Inf	0.017	0.988
Pre NO: *T. bernacchii - T. nicolai*	-0.1749	0.198	Inf	-0.882	0.528
Pre NO: *T. bernacchii - P. borchgrevinki*	1.0592	0.22	Inf	4.825	**0.001**
Pre NO: *T. pennellii - T. nicolai*	-0.1783	0.198	Inf	-0.900	0.522
Pre NO: *T. pennellii - P. borchgrevinki*	1.0558	0.22	Inf	4.797	**0.001**
Pre NO: *T. nicolai - P. borchgrevinki*	1.2341	0.218	Inf	5.656	**0.001**

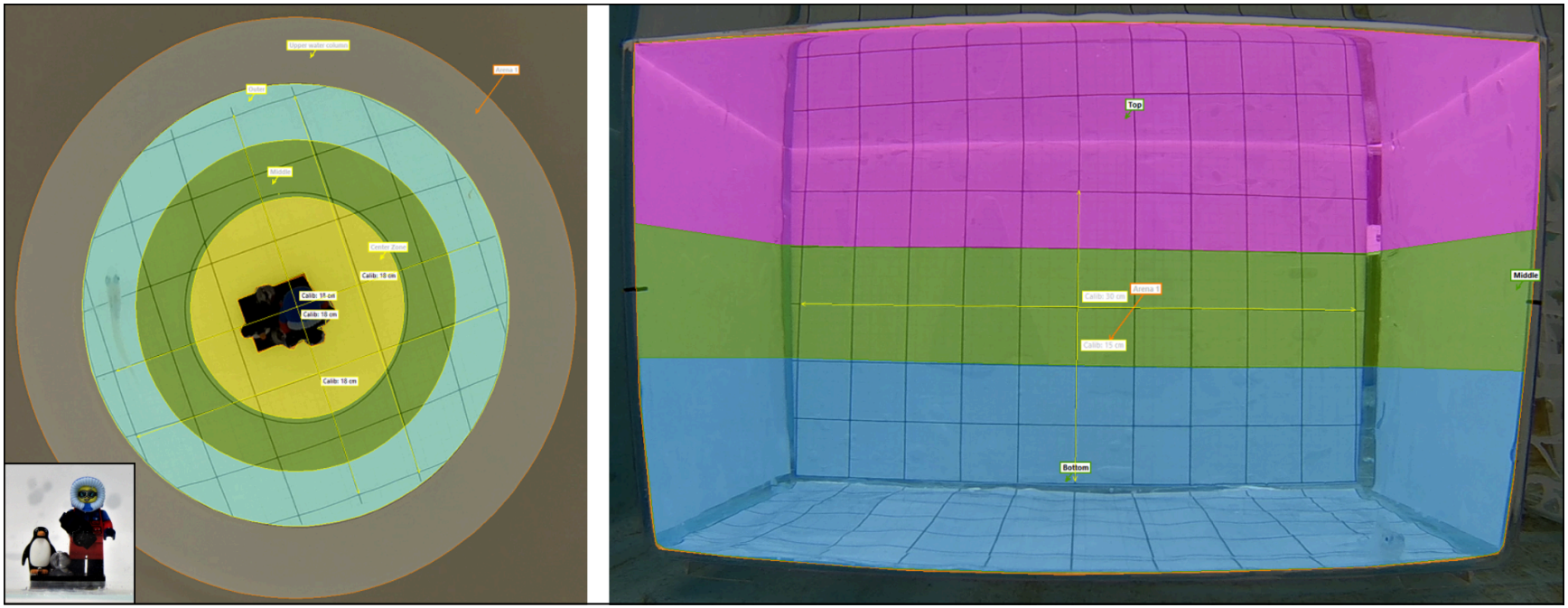
Post NO: *T. bernacchii - T. pennellii*	-0.4227	0.207	Inf	-2.038	0.118
Post NO: *T. bernacchii - T. nicolai*	-0.2643	0.209	Inf	-1.265	0.390
Post NO: *T. bernacchii - P. borchgrevinki*	0.0822	0.215	Inf	0.383	0.800
Post NO: *T. pennellii - T. nicolai*	0.1585	0.204	Inf	0.778	0.569
Post NO: *T. pennellii - P. borchgrevinki*	0.505	0.21	Inf	2.401	**0.051**
Post NO: *T. nicolai - P. borchgrevinki*	0.3465	0.211	Inf	1.641	0.209
Frequency Entering Outer Zone					
T. bernacchii: pre - post NO	0.439	0.121	Inf	3.626	**0.002**
T. pennellii: pre - post NO	0.219	0.118	Inf	1.859	0.143
T. nicolai: pre - post NO	0.121	0.12	Inf	1.010	0.486
P. borchgrevinki : pre - post NO	0.174	0.123	Inf	1.407	0.320
Pre NO: *T. bernacchii - T. pennellii*	-0.1678	0.154	Inf	-1.090	0.448
Pre NO: *T. bernacchii - T. nicolai*	0.1105	0.155	Inf	0.712	0.594
Pre NO: *T. bernacchii - P. borchgrevinki*	0.2899	0.157	Inf	1.852	0.143
Pre NO: *T. pennellii - T. nicolai*	0.2782	0.154	Inf	1.804	0.156
Pre NO: *T. pennellii - P. borchgrevinki*	0.4577	0.156	Inf	2.933	**0.015**
Pre NO: *T. nicolai - P. borchgrevinki*	0.1795	0.157	Inf	1.142	0.438
Post NO: *T. bernacchii - T. pennellii*	-0.388	0.158	Inf	-2.454	**0.048**
Post NO: *T. bernacchii - T. nicolai*	-0.2076	0.159	Inf	-1.306	0.377
Post NO: *T. bernacchii - P. borchgrevinki*	0.0248	0.161	Inf	0.154	0.918
Post NO: *T. pennellii - T. nicolai*	0.1804	0.156	Inf	1.154	0.436
Post NO: *T. pennellii - P. borchgrevinki*	0.4128	0.158	Inf	2.606	**0.035**
Post NO: *T. nicolai - P. borchgrevinki*	0.2324	0.159	Inf	1.459	0.295
Frequency Entering Wall Zone					
T. bernacchii: pre - post NO	0.52	0.164	Inf	3.169	**0.011**
T. pennellii: pre - post NO	0.756	0.173	Inf	4.372	**0.001**
T. nicolai: pre - post NO	0.453	0.168	Inf	2.689	**0.030**
P. borchgrevinki: pre - post NO	0.495	0.161	Inf	3.071	**0.011**
Pre NO: *T. bernacchii - T. pennellii*	0.0443	0.221	Inf	0.200	0.896
Pre NO: *T. bernacchii - T. nicolai*	0.2611	0.221	Inf	1.182	0.421
Pre NO: *T. bernacchii - P. borchgrevinki*	-0.1604	0.218	Inf	-0.736	0.594
Pre NO: *T. pennellii - T. nicolai*	0.2167	0.221	Inf	0.982	0.487
Pre NO: *T. pennellii - P. borchgrevinki*	-0.2047	0.219	Inf	-0.933	0.511
Pre NO: *T. nicolai - P. borchgrevinki*	-0.4214	0.219	Inf	-1.922	0.137
Post NO: *T. bernacchii - T. pennellii*	0.2806	0.228	Inf	1.230	0.396
Post NO: *T. bernacchii - T. nicolai*	0.1943	0.227	Inf	0.857	0.535
Post NO: *T. bernacchii - P. borchgrevinki*	-0.1848	0.223	Inf	-0.827	0.551
Post NO: *T. pennellii - T. nicolai*	-0.0863	0.229	Inf	-0.376	0.800

Post NO: *T. pennellii - P. borchgrevinki*	-0.4653	0.226	Inf	-2.055	0.115
Post NO: *T. nicolai - P. borchgrevinki*	-0.3791	0.225	Inf	-1.685	0.198
NOVEL TANK					
Mean Velocity					
T. bernacchii - T.pennellii	0.163	0.207	72	0.787	0.628
T. bernacchii - T. nicolai	0.161	0.257	72	0.627	0.743
T. bernacchii - P. borchgrevinki	0.025	0.238	72	0.106	0.938
T. pennellii - T. nicolai	-0.002	0.299	72	-0.005	0.996
T. pennellii - T. borchgrevinki	-0.138	0.282	72	-0.488	0.769
T. nicolai - P. borchgrevinki	-0.136	0.321	72	-0.424	0.785
Frequency Entering Top Zone					
T. bernacchii - T.pennellii	0.518	0.242	Inf	2.138	0.076
T. bernacchii - T. nicolai	0.401	0.248	Inf	1.616	0.186
T. bernacchii - P. borchgrevinki	-0.087	0.238	Inf	-0.366	0.811
T. pennellii - T. nicolai	-0.117	0.251	Inf	-0.466	0.769
T. pennellii - T. borchgrevinki	-0.605	0.242	Inf	-2.503	**0.032**
T. nicolai - P. borchgrevinki	-0.488	0.248	Inf	-1.971	0.098
Frequency Entering Bottom Zone					
T. bernacchii - T.pennellii	0.544	0.207	Inf	2.628	**0.025**
T. bernacchii - T. nicolai	0.120	0.210	Inf	0.573	0.743
T. bernacchii - P. borchgrevinki	-0.111	0.203	Inf	-0.548	0.743
T. pennellii - T. nicolai	-0.424	0.213	Inf	-1.988	0.098
T. pennellii - T. borchgrevinki	-0.655	0.207	Inf	-3.173	**0.008**
T. nicolai - P. borchgrevinki	-0.231	0.209	Inf	-1.106	0.452
Max Time in Top Zone					
T. bernacchii - T.pennellii	-4.86	5.78	72	-0.842	0.625
T. bernacchii - T. nicolai	24.88	4.42	72	5.635	**0.001**
T. bernacchii - P. borchgrevinki	17.44	5.11	72	3.415	**0.005**
T. pennellii - T. nicolai	29.75	4.71	72	6.316	**0.001**
T. pennellii - T. borchgrevinki	22.3	5.36	72	4.159	**0.001**
T. nicolai - P. borchgrevinki	-7.45	3.86	72	-1.93	0.111
Max Time in Bottom Zone					
T. bernacchii - T.pennellii	-0.503	0.222	72	-2.269	0.064
T. bernacchii - T. nicolai	-0.766	0.264	72	-2.900	**0.016**
T. bernacchii - P. borchgrevinki	-0.536	0.185	72	-2.894	**0.016**
T. pennellii - T. nicolai	-0.263	0.309	72	-0.851	0.625
T. pennellii - T. borchgrevinki	-0.033	0.247	72	-0.134	0.938
T. nicolai - P. borchgrevinki	0.230	0.286	72	0.804	0.628

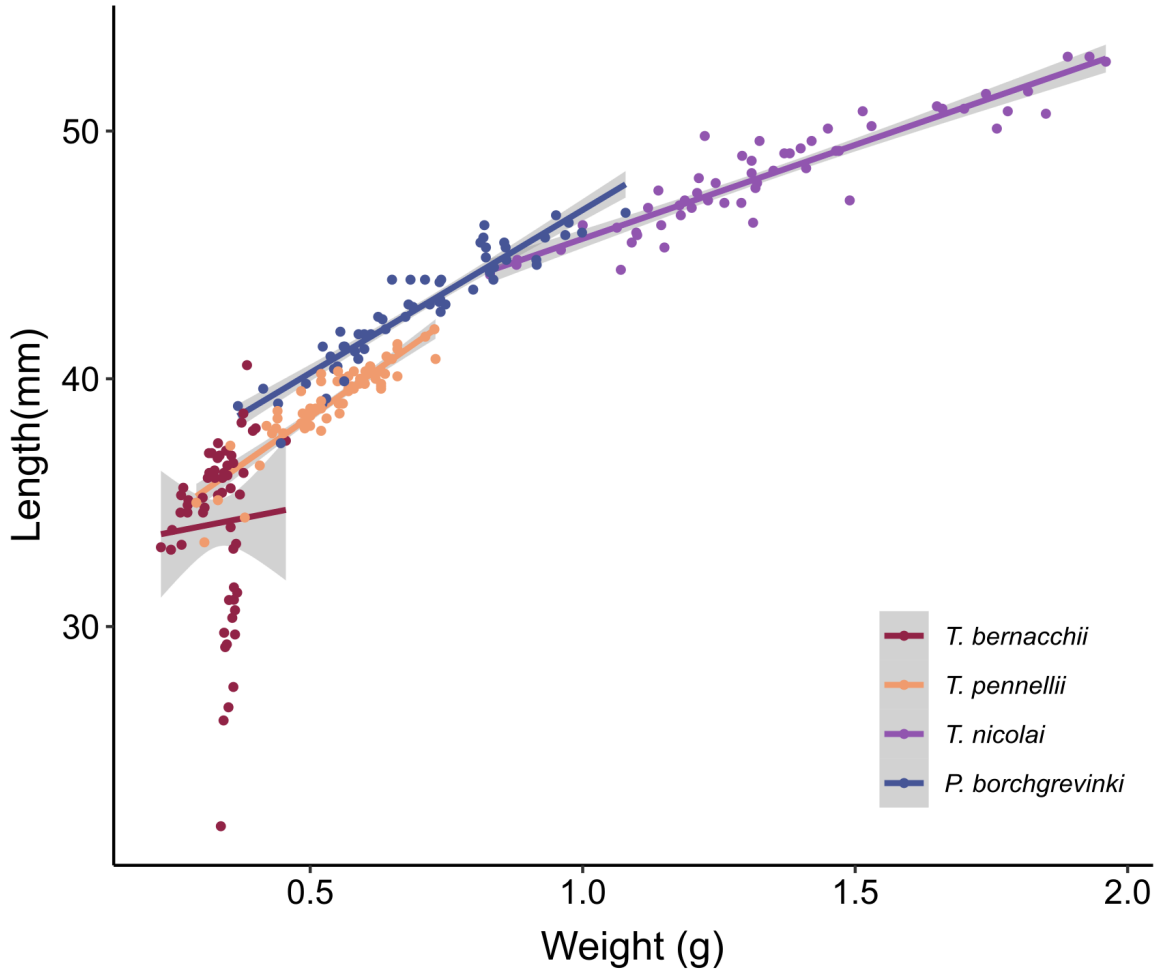
Percent Time in Top Zone					
T. bernacchii - T.pennellii	0.081	0.258	Inf	0.312	0.834
T. bernacchii - T. nicolai	1.879	0.326	Inf	5.764	**0.001**
T. bernacchii - P. borchgrevinki	0.525	0.258	Inf	2.035	0.093
T. pennellii - T. nicolai	1.799	0.309	Inf	5.817	**0.001**
T. pennellii - T. borchgrevinki	0.445	0.266	Inf	1.670	0.173
T. nicolai - P. borchgrevinki	-1.354	0.326	Inf	-4.148	**0.001**
Percent Time in Bottom Zone					
T. bernacchii - T.pennellii	-0.106	0.193	Inf	-0.548	0.743
T. bernacchii - T. nicolai	-0.687	0.205	Inf	-3.348	**0.005**
T. bernacchii - P. borchgrevinki	-0.651	0.199	Inf	-3.273	**0.005**
T. pennellii - T. nicolai	-0.581	0.205	Inf	-2.828	**0.016**
T. pennellii - T. borchgrevinki	-0.545	0.199	Inf	-2.737	**0.018**
T. nicolai - P. borchgrevinki	0.036	0.210	Inf	0.172	0.929

S. Table 5. Model summaries for Length x Weight linear regression for all species.

species	estimate	s.e.	F-statistic	df	adjusted R^2	p-value
T. bernacchii	4.27	11.02	0.2	55	-0.02	0.70
T. pennellii	15.39	0.93	275.1	59	0.82	<0.001
T. nicolai	7.58	0.42	330.1	53	0.86	<0.001
P. borchgrevinki	13.15	0.65	410.1	55	0.88	<0.001



S. Figure 1. Arena layouts for the Novel Object Test (NOT, left) and the Novel Tank Test (NTT, right). For the NOT, zones include the Wall Zone in gray, the Outer Zone in teal blue, the Middle Zone in green, and the Inner Zone in yellow. The novel object (left, inset box) is outlined in the center of the arena. For the NTT, zones include the Top Zone in pink, the Middle Zone in green, and the Bottom Zone in blue.



S. Figure 2. Linear regression of Length (mm) by Weight (g) of juvenile *T. bernacchii* (maroon, n=57), *T. pennellii* (orange, n=61), *T. nicolai* (purple, n=55), and *P. borchgrevinki* (blue, n=57). Gray bands denote the 95% confidence interval for each linear regression.

CHAPTER 3

Acclimation to ocean warming and acidification conditions alter exploratory-avoidant behavior in two juvenile Trematomus fishes in McMurdo Sound, Antarctica

Abstract

The Southern Ocean is experiencing climate change at a rate among the fastest on Earth, and it is thought that endemic, small-bodied, low-dispersing, benthic Antarctic marine organisms may be particularly vulnerable due to their specialization to this unique, stenothermal habitat. Ocean warming (OW) does not occur in isolation, and concurrent ocean acidification (OA) from excess dissolved carbon dioxide (pCO_2) is also threatening marine ecosystems and species globally. Current literature on the effects of OAxOW on Antarctic fishes is limited to a handful of studies, primarily centered on physiological and metabolic responses to stress, with sparse behavioral components and limited information on early life stages. We still have no experimental data on most Antarctic fish species, and we lack information on how inter- and intra-specific behavioral strategies and larger ecological dynamics (e.g., predator-prey interactions, species coexistence, habitat use) might impact species and community resilience to climate change. Behavior is the first response to potential stressors, and understanding interspecific behavioral responses to environmental stressors gives important information on species-specific performance. Fishes around the world – including one Antarctic species – have demonstrated behavioral responses to OAxOW stressors, especially when examined concurrently. In this study, we examined the interactive effects of OW (–1°C, +2°C) and ocean acidification (ambient (~475), moderate (~800), and high (~1050) µatm pCO_2) on the behavior of two closely related Notothenioid fishes,

Trematomus bernacchii and *Trematomus pennellii*. We focused on the exploratory-avoidant axis of behavior using the Novel Object Test (NOT) to assess if warming and carbon dioxide (pCO_2) impact exploratory activity or induce anxiety and/or neophilia (i.e., interest in novel objects). Over five weeks of acclimation, warming was the primary driver of behavioral change in both species. Elevated pCO_2 did not significantly alter *T. pennellii* behavior and only showed subtle effects in *T. bernacchii* that dissipated early within the acclimation period. Although OAxOW interactions were not significant, observed trends suggest potential interactive effects of OAxOW that warrant further investigation. In both species, fishes acclimated to +2°C demonstrated reduced mean velocity (cm s^{-1}), a metric of exploratory activity, and increased their total time in the center zone (s) of a testing arena, an indication of neophilia. These responses amplified over time, and *T. pennellii* demonstrated a stronger response (i.e., effect sizes) in both behavioral metrics. *T. bernacchii* showed modest growth over the acclimation period, but *T. pennellii* did not, indicating different growth trajectories that may be important for community dynamics (e.g., coexistence) and life history strategies. Consistent with previous physiological and behavioral studies, while limited, our results support the inference that *T. pennellii* have a particularly risky strategy when faced with novelty that is amplified when acclimated to warming. It is currently unclear if a riskier strategy is adaptive or maladaptive in this ecosystem.

Introduction

The world's oceans are the regulators of global climate, and buffer climate change by taking up excess atmospheric carbon dioxide (CO_2) and absorbing the associated excess heat (IPCC, 2023). In recent decades, oceans have absorbed 90% of excess heat from climate change, with polar oceans accounting for disproportionately high rates of heat and CO_2 uptake (IPCC, 2019). The Southern Ocean alone accounts for approximately 40% of the total CO_2 uptake by oceans globally (Frölicher *et al.*, 2015; Gruber *et al*., 2019). Under the Intergovernmental Panel on Climate Change high-emission SSP5-8.5 scenario (business-as-usual), global mean sea surface temperatures (SST) are projected to rise by approximately 2.9°C (with a likely range of 2.0°C to 4.1°C) by the end of the 21st century (1995–2014 to 2081–2100; IPCC CMIP6 AR6). The Antarctic is also warming at a rate 0.5°C higher than the global average, the second highest rate globally after the Arctic (IPCC, 2019). Critically, the rate of warming is also accelerating (IPCC, 2021). Warming does not occur in isolation, and concurrent acidification from excess dissolved CO_2 is also threatening marine ecosystems and species globally (IPCC, 2023). The deleterious effects of warming on marine organisms and ecosystems, including those in the Antarctic, are relatively well described (Beers and Jayasundara, 2015; Bilyk and DeVries, 2011; Todgham and Mandic, 2020), but much less is known regarding effects of CO_2, especially when examined with warming (although see: Davis *et al*., 2018; Enzor *et al*., 2013; Enzor *et al*., 2017; Enzor and Place, 2014; Naslund *et al.*, 2021).

Antarctic species and ecosystems are thought to be particularly vulnerable to climate change, not only because they are experiencing climate change at among the

fastest rates on Earth, but also because of their unique evolutionary history and specialization to life in the Antarctic. The opening of the Drake Passage and corresponding strengthening of the Antarctic Circumpolar Current (ACC) approximately 10-14 mya effectively isolated small-bodied species surrounding Antarctica from the rest of the world (Barker and Thomas, 2004; Livermore *et al*., 2005). The strengthening of the ACC rapidly cooled and glaciated the Antarctic, resulting in mass extinction (Scher and Martin, 2006; Scher *et al*., 2015). The few species that did persist through this transition then diversified. Subsequently, many of the few examples of marine species flocks (monophyletic radiations of closely related species) are found in the Southern Ocean and include Notothenioid fishes (Bowen *et al*., 2020; Eastman and McCune, 2000). The habitat in which these species evolved is stenothermal, and remains around -1.9°C nearly year-round in southern high-latitude waters, although occasional temperature spikes up -0.4°C have been recorded in the austral summer (Cheng and Detrich, 2007; Colombo *et al.*, 2015; Cziko *et al.*, 2020; Eastman, 2005). More thermal fluctuation is seen on the West Antarctic Peninsula (WAP), where temperatures can regularly reach up to ~3°C in the austral summer, but this thermal regime is still quite narrow in comparison to temperate ecosystems (Meredith and King, 2005). More recently, rare temperature extremes upwards of 10°C have been recorded on the WAP (Gorodetskaya *et al.*, 2023). Cold water also holds more dissolved gases, including oxygen and carbon dioxide (Emerson and Hedges, 2008). Compared to more temperate regions, the Antarctic experiences greater fluctuations in pCO_2, due to sea ice phenology and boom/bust seasonal productivity cycles (Clarke, 1988; Kapsenberg *et al*., 2015; Jones *et al*., 2023; Roden *et al*., 2016). Low pCO_2 events are projected to

lengthen in McMurdo Sound and may put marine organisms – including sensitive early life stages – in extended periods of acidification stress (Flynn *et al*., 2015; Ishimatsu *et al*., 2004; Kapsenberg *et al.*, 2015; Pörtner and Peck, 2010).

Across taxa, Antarctic organisms show specialized physiological adaptations to life in Antarctic waters, including more flexible membranes, altered enzyme kinetics, mitochondrial proliferation, antifreeze glycoproteins, and the loss of some inducible heat shock proteins (Bista *et al.*, 2023; Cheng and Detrich, 2007; Daane and Detrich, 2022; Somero, 2004; Somero *et al.*, 1998; Todgham and Mandic, 2020; Verde *et al*., 2011). Many species also exhibit slow paces of life, with delayed and extended life history strategies (Agüera *et al.*, 2015; La Mesa and Vacchi, 2001; Sol *et al.*, 2025; Peck, 2002). While these features are thought to be adaptive (or neutral) in the Antarctic waters, it is thought that these attributes may malfunction and become maladaptive as oceans change, particularly as temperatures rise significantly above freezing (Montgomery and Clements, 2000; Todgham and Mandic, 2020). While it is possible that a modest amount of warming could relieve the significant energetic cost of freeze avoidance and growth and metabolism at low temperatures (Fraser *et al*., 2022; Naslund *et al*., in review; Peck, 2002), any energy savings could be offset by coping with other stressors associated with warming. Because of the long life histories of many polar species, the rapid rates of change, and the fact that there is nowhere colder to go, Antarctic species are limited in their adaptive capacity to respond to rapid climate change; they must rely on whatever existing physiological and behavioral plasticity they already have (Byrne *et al.*, 2020; Byrne and Lamare, 2024; Peck, 2018; Todgham and Mandic, 2020).

Studies examining how Antarctic fishes may cope with climate change have focused primarily on effects of temperature (Beers and Jayasundara, 2015; Bilyk and DeVries, 2011; Todgham and Mandic, 2020), but research has consistently shown that co-occurring stressors usually interact non-additively, and can be synergistic or antagonistic (Breitburg *et al*., 2015; Crain *et al*., 2008; Darling and Côté, 2008; Hofmann and Todgham, 2010; Todgham and Stillman, 2013). As such, it is difficult to accurately predict species responses to co-occurring stressors without studying them in concert. Of particular concern for marine species is CO_2 reacting with water molecules as it dissolves, increasing the concentrations of free hydrogen ions (H+) and acidifying the water (Doney *et al*., 2009). The surplus of H+ react with carbonate (CO_3^{2-}), forming bicarbonate (HCO_3^{2-}) and lowering the normally abundant availability of free carbonate. For calcifying organisms, such as hard corals, mollusks, and pteropods, that rely on free carbonate to form their calcium carbonate skeletons and shells, acidification could be catastrophic (Bednaršek *et al*., 2012; Guinotte and Fabry, 2008; Kroeker *et al*., 2013; Orr *et al*., 2005). Moderate acidification often impedes growth, weakens shells and skeletons, and increases the frequency of deformities and abnormalities, which in turn can increase vulnerability to predation and other environmental stressors (Figuerola *et al*., 2021; Hofmann *et al*., 2010; Kroeker *et al*., 2013; Medeiros and Souza, 2023). High levels of acidification can cause direct dissolution of existing calcium carbonate structures and can lead to widespread mortality (Andersson and Gledhill, 2014; Bednaršek *et al*., 2012).

Fishes are also at risk and have been shown to demonstrate strong behavioral responses to pCO_2 that could impact foraging, predator-detection, and habitat use

behavior (Briffa *et al*., 2012; Clements and Hunt, 2015; Tresguerres and Hamilton, 2017). Acidification was thought to deteriorate otoliths (calcified ear-bones), but contrary to expectations, pCO_2 often has the opposite effect and increases otolith growth, especially in larval life stages (Bignami *et al.*, 2013; Checkley *et al*., 2009; Munday *et al.*, 2008). Otoliths play important roles in sensory processes, movement, and hearing, and altered otolith morphology has been shown to negatively impact function, including hearing sensitivity and directional orientation (Radford *et al.*, 2021). In juvenile *T. bernacchii*, warming but not pCO_2 affected otolith growth (Naslund *et al*., 2021). Acidification also has been shown to affect many facets of fish behavior in more temperate species, including impaired predator-avoidance and risk-prone behavior, sensory cue responses, altered activity and foraging behaviors, and social and learning deficits (Briffa *et al*., 2012; Dixson *et al.*, 2010; Clements and Hunt, 2015; Tresguerres and Hamilton, 2017). The research topic of the impacts of pCO_2 on fish behavior remains contentious (Alter *et al.*, 2024; Browman, 2016, 2017; Clark *et al*., 2020a, 2020b; Clements *et al*., 2022; Esbaugh *et al*., 2023; Munday *et al*., 2020), but, generally, pCO_2 can have significant effects – most often with small effect sizes – on fishes, especially in concert with OW, but not always (Sundin, 2023). Understanding how species cope with co-occurring stressors is essential for evaluating species vulnerability to climate change.

Physiological adaptations to survive the Antarctic have received considerable attention, perhaps due to our anthropocentric curiosity to make sense of how ectotherms could possibly survive a below freezing environment (Cziko *et al.*, 2006; DeVries and Cheng, 2005; Eastman and DeVries, 1985). Behavioral adaptations to

survive this habitat, though, are poorly described. Behavior is just as important as physiology when coping with potential environmental and ecosystem stressors (Moiron *et al*., 2020; Wong and Candolin, 2015). Behavior is the “key first response” to potential stressors (Sih, 2012), and appropriate responses to many potential stressors faced by Antarctic fishes, such as increased invasions by more temperate species, will have strong behavioral components (Chown *et al*., 2015; Duffy *et al*., 2017; Rintoul *et al*., 2018). Behavior drives inter- and intra-specific dynamics that affect community and population stability, including competition, predator-prey interactions, and coexistence (Preisser and Orrock, 2012; Sih, 1987). There are extremely few studies focusing on Antarctic fish behavior (Ferrando *et al*., 2014; Ismailov *et al*., 2021), only two of which focus on early life stages (Davis *et al*., 2018; Naslund *et al.*, in review).

In this study, we examined how two abundant, closely related species in McMurdo Sound (*Trematomus bernacchii* and *Trematomus pennellii*) may respond to future climate change stressors. While *T. bernacchii* are relatively well-studied, there has been much less research examining *T. pennellii*. Based on previous work examining co-occurring stressors in fishes generally, as well as limited previous studies on Antarctic fishes, we hypothesized that warming would be the dominant driver of behavioral and growth responses in both *T. bernacchii* and *T. pennellii*, but that elevated pCO_2 would modulate these effects through non-additive interactions. While research on *T. pennellii* is sparse – and nonexistent for OAxOW stressors, the limited information we do have on *T. pennellii* suggests that juvenile *T. pennellii* may be more risk-prone, have a more flexible diet, and tolerate higher temperatures than *T. bernacchii* (Chapter 2; Brenner, 2001; Mandic *et al.*, 2022; Mark *et al.*, 2005; McMullin *et al*., 2017; Naslund *et*

al., in review; Podrabsky and Somero, 2006). As such, we predicted that *T. pennellii* acclimated to elevated OAxOW would exhibit riskier strategies, including higher exploratory activity and greater interest in novelty, or neophilia. We anticipated OAxOW would lower growth rates in both species. Even closely related species often show different responses to climate change stressors, so examining interspecific responses to concurrent stressors – especially at early life stages – is essential when assessing species and ecosystem vulnerability and potential resilience.

Materials and Methods

1. Experimental Design, pCO_2 System, and Seawater Chemistry

Experiments were conducted at McMurdo Station in the Ross Sea, Antarctica in the austral summers of 2018, for *T. bernacchii*, and 2019, for *T. pennellii*. Our experimental system for *T. bernacchii* consisted of a 2×3 factorial design, with two levels of temperature (-1°C and +2°C) and three levels of pCO_2 (ambient: ~475 µatm; moderate: ~800 µatm; and high: ~1,050 µatm). Treatment levels were chosen to span current conditions and the conservative lower bound of late-21st Century warming projections under the 'business-as-usual' uncurbed, high-emissions RCP8.5 ensemble projection (ΔT = +3.7°C; likely range 2.6 – 4.8°C; $pCO_2 \approx$ 936 µatm by 2100; IPCC AR5 WG1 SPM, 2014: Table SPM.2 & Box SPM.1). Fishes were acclimated in their OA×OW treatments for five weeks. *T. bernacchii* were sampled at 1, 2, and 5 weeks. Due to limited numbers, *T. pennellii* were only sampled at 2 and 5 weeks, with a smaller control treatment of -1°C and ambient pCO_2 at 0 weeks (when experiments started). Likewise,

we removed the moderate pCO_2 level, so the treatments for *T. pennellii* were limited to a smaller 2×2 factorial design.

Experimental pCO_2 levels were simulated using a pCO_2 gas delivery system as described in Davis *et al.*, (2018). Briefly, three mixtures of scrubbed air and CO_2 gas were maintained with mass-flow controllers (Sierra Instruments, USA) and continuously delivered via venturi injector into 19 L reservoirs of fresh seawater pumped from McMurdo Sound. Each of the nine 19 L culture buckets (three replicates per treatment) was drip-fed the reservoir water at 16 L h^{-1}. The same mixtures of scrubbed air and CO_2 gas were also directly bubbled into each culture bucket at 1.02 L min^{-1} to maintain target pCO_2 and ensure fully oxygenated water for the animals.

Experimental temperatures were maintained by placing the reservoir and culture buckets in 680 L square aquaria. The cold water bath was the ambient temperature of the seawater pumped in from the sound (-1.3 ± 0.1°C). The warm water bath was maintained with heat bars and temperature controllers. We recorded the temperature of the water bath, each culture bucket, and the incoming seawater every morning with a handheld thermocouple and with submerged HOBO data loggers (Onset, USA) set to record every 30 min.

Seawater chemistry was closely monitored for the entire duration of the experiment. We measured the total pH of every reservoir and culture bucket every two days via spectrophotometer (Shimadzu) and m-cresol dye protocol (SOP 6b, Dickson *et al*., 2007). Every four days, we measured the total alkalinity (TA) of the reservoir buckets using open-cell titration (T50 titrator, Mettler Toledo, USA) with a certified reference material (CRM) standard and titrant (Dickson Laboratory, Scripps Institute, USA) (SOP

3b, Dickson *et al*., 2007). We also measured the salinity every four days using a conductivity meter (YSI, USA). Thus, every other day we had actual pCO_2 levels of every bucket, which were calculated via 'SeaCarb' (Lavigne *et al*., 2011), and we confirmed stable pCO_2 levels in each treatment. Actual seawater chemistry recordings are reported in **Table 1**.

2. Fish Collection, Care, and Maintenance

T. bernacchii were collected daily October 30 through November 2, 2018 at Cape Evans Ice Wall (77°38′23.8″S, 166°31′09.7″E). *T. pennellii* were collected daily October 21 through October 29, 2019. Because *T. pennellii* are less abundant, we collected *T. pennellii* at Arrival Heights (77°50'31.7"S, 166°38'10.6"E) and the intake jetty (77°51.076'S, 166°39.852'E), in addition to Cape Evans Ice Wall, to obtain sufficient numbers of individuals. The jetty and Arrival Heights are close to McMurdo Station, ~1 km apart, while Cape Evans Ice Wall is ~23 km to the north. All fishes were carefully hand-collected by SCUBA divers and promptly transported to the aquarium room in the A.P. Crary Science and Engineering Center, as described in **Chapter 2**.

Fishes were visually inspected for injuries, separated by species, counted, and held at ambient temperature (approximately -1.3°C) for at least one day but no more than four days (for *T. bernacchii*) and eight days (for *T. pennellii*) before being separated into experimental treatments. Species were identified based on morphological differences as described in **Chapter 2**. We were not able to determine sex and assumed fishes were in their second year of development, as described in previous studies (Davis *et al*., 2016; Davis *et al*., 2018; La Mesa *et al*., 1996; North, 1991). Fishes were fed approximately 0.03g/fish of frozen plankton (Hikari Bio-Pure Ocean

Plankton, Hayward, CA, USA) twice daily throughout the acclimation period. Fishes were fasted overnight prior to experiments to minimize any effects of digestion. Acclimation tanks were siphoned once daily in the evening to maintain water quality while limiting fish disturbance. We monitored water quality throughout the duration of the experiment to confirm that the flow-through was sufficient to flush out dissolved nitrogenous waste. We measured the dissolved nitrate, nitrite, and ammonia levels using commercial water quality test kits (API, USA). Nitrate, nitrite, and ammonia levels were never above zero. All research was conducted in accordance with US federal animal welfare laws by approval by the University of California Davis Institutional Animal Care and Use Committee (protocol no. 20558).

3. Novel Object Test

Novel Object Tests were performed following a modified protocol from Hamilton *et al.* (2014) and Hamilton *et al.* (2017), as described in **Chapter 2**. Circular buckets (20 cm diameter) with 1 x 1 cm grids affixed to the bottom were filled to 6 cm with treatment seawater (i.e., ambient, moderate, or high pCO_2 and -1 or +2°C) and placed in a water bath to maintain treatment temperature. Four fishes were tested concurrently, and for every trial we mixed treatments to minimize any random effects of the time of day or trial number. Individuals from the corresponding treatment were gently and quickly transferred to the arenas and lids with affixed action cameras (Activeon CX Action Camera, United States) were placed on top of the arena. Baseline fish behavior was recorded for ten minutes. Subsequently, the lids were gently removed, novel objects were carefully placed into the center of the arena, and lids were replaced. LEGO ‘Female Wildlife Photographer’ Minifigures were used as the novel object as they were

an appropriate size compared to the fishes, displayed colors across the spectrum, and included complex shapes. Fish behavior with the novel object present was then recorded for ten minutes. We left the aquarium when experiments were running, apart from placing the novel objects, which we did efficiently and quietly to minimize disturbance as much as possible (SAMPLE SIZE).

All video recordings were analyzed using EthoVisionXT v17.4 (Noldus, NL) video tracking software at the University of California, Davis. We used QuickTime Player to split each video into halves, the first half being the control (‘pre’) and the second half with the novel object (‘post’). A unique arena was designated for every video half due to slight variations in camera angles from observers replacing the lids following placement of the novel object. The arenas were designated as the Wall, Outer, Middle, and Center zones and the novel object was marked as space impossible for the fish to occupy (**Chapter 2, Appendix S. Figure 1**). We set the zone exit threshold as 1.0 cm and tracked the center-point of the fish using contour-based detection. The first 30 seconds were removed; we did not see any patterns in their movement over the time of the trial to suggest that removing more time was necessary for the fishes to settle.

After data acquisition, we watched every recorded trace to ensure the fish was accurately tracked as recommended in Khursigara *et al*. (2023). When tracks were poor, we reran the acquisition of that trial with unique detection settings optimized for that specific fish. When tracks had minor missing segments or the software identified something other than the fish as the fish, we manually fixed these errors using the Interpolate tool and by dragging the Center Point dot to the correct location on the fish.

Statistical outliers were not removed, but one individual was removed per species due to equipment malfunction for a final N=215 for *T. bernacchii* and N=107 for *T. pennellii*.

Metrics of interest were selected *a priori*, based on literature and the known behavioral ecology of these species, as well as *posteriori* via Principal Component Analysis (PCA). The PCAs were performed in R using the R Studio interface (v4.4.1, R Core Team, 2024) as described in **Chapter 2** using the "factoextra" and "FactoMineR" packages (Kassambara and Mundt, 2020; Le *et al.*, 2008). The variables selected were the mean velocity (cm/s) and time spent in the center zone (s), which give insight into exploratory activity and interest in novelty (Maximino *et al*., 2010).

4. Morphometrics

After NOTs were complete, fishes were immediately sacrificed in a lethal dose of tricaine methanesulfonate (500 mg/L, MS-222) prepared in fresh seawater. Fish were then gently dabbed dry with Kimwipes, immediately weighed to the nearest 0.01 g, and the standard length measured using calipers to the nearest 0.1 mm. Fulton's condition factor (CF) was calculated as:

$$Condition\ Factor\ = \frac{Weight\ (g)}{Total\ Length\ (cm)^3} \times 100$$

5. Statistical Analyses

All statistical analyses were conducted in R using the R Studio interface using linear mixed effects models via "lme4" (Bates *et al*., 2015). The only exception was the total time spent in the center zone, which was heavily right-skewed and more appropriately fit by a generalized mixed-effects model (GLMM) in "glmmTMB" using a log-linked

Tweedie distribution (Brooks *et al*., 2017). Models were inspected for linearity, homoscedasticity, independence, and normality via visual inspection of fitted values vs. residuals, lag plots, QQ plots, and histograms of model residuals, as advised in Zuur *et al*. (2010). Models were built via biologically informed backwards model selection as advised in Harrison *et al*. (2018) and Zuur *et al.* (2009).

To determine model structure, we first fit the full mixed-effects model with a four-way interaction of all fixed effects ('novel object', 'week', 'temperature', and 'pCO_2') for NOT models and a three-way interaction of all fixed effects ('week', 'temperature', and 'pCO_2') for morphometric models. We then evaluated nested models against the reference model with an ANOVA (via "stats") using restricted maximum likelihood testing (REML) for random effects and maximum likelihood testing for fixed effects. We compared the model fits via AIC, BIC, log-likelihood, and p-values (**Appendix S. Table 1**). The random effect of 'arena' (i.e., which of the four buckets the fish was tested in for NOT) did not have an effect on any NOT model, worsened model fit, and added unnecessary complexity. As such – and because it is a momentary testing environment rather than a true hierarchical component of the experimental design – we removed 'arena' from all NOT models. The random effect of 'fish ID' was retained in all NOT models, because each individual fish has a 'pre' and 'post' novel object observation. 'Culture bucket' did not have a significant effect on any model, but we retained it in all morphometric and NOT models because it is inherent nesting in our experimental design.

After testing the random effects, we determined the fixed effects structure by first examining the four-way interaction (for NOT models), then all three-way interactions,

then all two-way interactions. At each step, any non-significant highest-order interaction was dropped and the next set of interactions was tested. Additive fixed effects of 'week', 'temperature', and 'pCO_2' were retained in all reported final models to respect our experimental design, even if they did not have a significant effect at the α = 0.05 significance-level. Accordingly, in all NOT models, 'novel object' ('pre', before the object was added; 'post', after the object was added) was always included as a fixed effect. Final models were refitted by REML for parameter estimation and interpretations.

For all models, the "emmeans" package was used to calculate pairwise comparisons of means across species, without adjustment (Lenth, 2024). We adjusted p-values for false discovery rate using the Benjamini-Hochberg correction (via "stats"). All contrasts are available in **Appendix S. Table 1.** An α of 0.05 was used as the significance level for all statistical tests. We calculated the conditional and marginal R^2 for each model to evaluate model fit and variance explained by fixed vs. random effects via the "MuMIn" and "performance" packages (Bartón, 2024; Lüdecke *et al*., 2021), as recommended in Nakagawa and Schielzeth (2013). For NOT models, we also calculated the Intra-class coefficients (ICC) for each model (via "performance") to understand the degree of repeatability of individuals. Data visualization was conducted using the "ggplot2" package (Wickham, 2016) and figure design in Inkscape (v1.3.2, Inkscape Team, 2023).

While our ultimate aim was to understand whether *T. bernacchii* and *T. pennellii* might respond differently to expected climate change, limitations to our experimental design (due to inherent logistical challenges of Antarctic fieldwork) preclude direct statistical contrasts between species. As such, we modeled each species' response to

OA×OW stressors independently and present modeling results in species-specific order – first *T. bernacchii*, then *T. pennellii*. All model selection comparisons, best-fit model summaries, and adjusted contrasts can be found in **Appendix S. Table 1**. Because culture bucket and fish ID (for NOTs) were included as random intercepts – and did not have any effects – they are not discussed below, although they are reported in **Appendix S. Table 1**.

Results

Trematomus bernacchii

Morphometrics

Weight

Our best fit model of *T. bernacchii* weight (g) was a linear mixed-effects model with additive effects of warming, pCO_2, and week. pCO_2 was retained in the model due to experimental design, but neither overall pCO_2 nor its interactions with week or temperature significantly improved model fit (all $p > 0.6$). However, moderate pCO_2 did drive a small, but significant, weight gain relative to the ambient group (est = 0.04 g, $t(209) = 2.38$, $p = 0.02$). High pCO_2 did not have an effect on *T. bernacchii* weight. Although not a significant effect in the overall model, there was also a small interactive effect of warming and acclimation time; between weeks 1 and 5, *T. bernacchii* in the +2°C treatment group gained an extra ~1/10th g compared to those in the –1°C treatment group (est = 0.07, $t(207)=2.35$, $p=0.02$). Acclimation time was the only significant main effect driving *T. bernacchii* weight. Fish grew modestly over the

acclimation period (1–5 wk est = 0.06 g, $t(195) = 3.91$, $p = 0.0001$; 2–5 wk est = 0.04 g, $t(195) = 2.53$, $p = 0.01$).

Standard Length

Our best fit model of *T. bernacchii* standard length (mm) was a linear mixed-effects model with additive effects of warming, pCO_2, and week. Neither pCO_2 nor temperature influenced *T. bernacchii* length, although they did grow considerably over the acclimation period (1–5 wk est = 0.77 mm, $t(195) = -2.83$, $p = 0.005$; 2–5 wk est = 0.65 mm, $t(195) = -2.40$, $p = 0.02$).

Fulton's Condition Factor

Our best fit model of Fulton's condition factor (CF) in *T. bernacchii* was a linear mixed-effects model with the interaction of week and warming, plus the fixed effect of pCO_2. Warming had a strong effect on CF that varied over the acclimation period; *T. bernacchii* acclimated in +2°C had lower CF than those at –1°C (est = –0.05, $t(207) = -2.40$, $p = 0.02$), but higher CF at week 5 (1–5 wk est = 0.09, $t(193) = -3.99$, $p = 0.0001$; 2–5 wk est = 0.05, $t(193) = -2.46$, $p = 0.01$). In contrast, CF remained stable across weeks in the –1°C treatment group (1–5 wk est = 0.01, $t(193) = 0.52$, $p = 0.60$). pCO_2 also had a main effect that was inconsistent; CF was higher in moderate pCO_2 levels relative to ambient (est = 0.04, $t(207) = 2.53$, $p = 0.01$), but not in the high pCO_2 treatment.

Novel Object Test

Mean Velocity

Our best fit model of mean velocity (cm s^{-1}) in *T. bernacchii* was a linear mixed-effects model with the two-way interaction of pCO_2 and week, plus the three-way interaction of week, warming, and novel object. There was a significant main effect of pCO_2 at week 1, where exposure to both moderate and high pCO_2 decreased mean velocity compared to ambient (mod est = -0.54 cm s^{-1}, t = -2.22, p = 0.03; high est = –0.68 cm s^{-1}, t = –2.46, p = 0.02). pCO_2 also shaped how velocity changed over the five-week acclimation: under ambient pCO_2, *T. bernacchii* slowed from week 1 to week 2 (est. = –0.73 cm s^{-1}, $t(242)$ = –2.31, p = 0.02), but recovered by week 5 (est. = 0.30 cm s^{-1}, p = 0.25). Under moderate and high pCO_2 levels, though, *T. bernacchii* swam faster on average from week 1 to week 2 (mod pCO_2 est = 0.87 cm s^{-1}, t = 2.38, p = 0.02; high pCO_2 est = 0.72 cm s^{-1}, t = 1.97, p = 0.05) and from week 1 to week 5 (mod pCO_2 est = 0.98 cm s^{-1}, t = 2.67, p = 0.008; high pCO_2 est = 1.05 cm s^{-1}, t = 2.89, p = 0.004). Between 2 and 5 weeks, this trend was only significant in the high pCO_2 treatment (est = 0.69 cm s^{-1}, $t(189)$ = 2.67, p = 0.008).

Across all treatments and weeks, *T. bernacchii* dramatically reduced their mean velocity in response to the novel object (–0.81 cm s^{-1}, t = –5.11, $p < 0.0001$), and this effect was amplified by warming and over the acclimation period. At week 2, *T. bernacchii* in the +2°C treatment group swam >1.5x slower in response to the novel object than those in the –1°C treatment group (est = 0.58 cm s^{-1}, $t(110)$ = 2.29, p = 0.02) and at week 5, twice as slow (est = 1.35 cm s^{-1}, $t(108)$ = 5.37, $p < 0.0001$). There were strong synergistic effects of warming and acclimation time: at week 2, *T. bernacchii* in the +2°C treatment group slowed by an additional ~10% (than would be expected for

only additive effects), and at week 5, they slowed by ~45% (2wk est = -0.75 cm s^{-1}, $t(212) = -2.36$, $p = 0.02$; 5wk est = -0.94 cm s^{-1}, $t(209) = -2.96$, $p = 0.003$). Warming also showed a significant main effect, where *T. bernacchii* acclimated to the +2°C treatment swam about half as fast as those acclimated to –1°C (–0.54 cm s^{-1}, $t = -2.15$, $p = 0.03$).

Total Time in Center Zone

Our best-fit model of total time *T. bernacchii* spent in the center zone (s) was a GLMM (log link) with a three-way interaction among novelty, warming, and week. pCO_2 was retained as a fixed effect, but was not a significant predictor of duration spent by *T. bernacchii* in the center zone ($p = 0.66$, LRT). Warming alone had no significant main effect on duration spent by *T. bernacchii* in the center zone (est. ratio = 1.36, $z = 1.18$, $p = 0.24$). However, *T. bernacchii* exhibited a strong response to the novel object (est. ratio = 1.97, $z = 3.15$, $p = 0.002$), and the magnitude of the response was dependent on week and temperature treatment. At week 1, *T. bernacchii* in both temperature treatments showed a similar response to the object by drastically increasing the amount of time in the center zone after the object was introduced (est. ratio = 0.67, $z = -1.93$, $p = 0.05$); *T. bernacchii* in the –1°C treatment group spent nearly double the amount of time in the center zone after the object was introduced (est. ratio = 0.51, $z = -3.15$, $p = 0.002$); for the +2°C group, *T. bernacchii* increased their time in the center by about 1.5x (est. ratio = 0.67, $z = -1.93$, $p = 0.05$). At week 2, only *T. bernacchii* in the –1°C group significantly increased time spent in the center (est. ratio = 0.59, $z = -2.51$, $p = 0.01$). The +2°C group showed this trend, but it was not significant (est. ratio = 0.75, $z =$

–1.48, p = 0.14). By week 5, this response only persisted – and amplified – in the +2°C group, which increased time in the center zone by >2.5x (est. ratio = 0.39, z = –4.61, p < 0.0001). For *T. bernacchii*, the synergistic interactions of warming, week, and novelty only emerged at week five, when they increased their time spent in the center zone by nearly 3x more than would be predicted by additive effects of warming and novelty (est. ratio = 2.78, z = 2.37, p = 0.02).

Trematomus pennellii

Morphometrics

Weight

Our best-fit model of *T. pennellii* weight (g) was a linear mixed-effects model with fixed effects of warming, pCO_2, and acclimation week. None of these predictors significantly influenced weight and *T. pennellii* weight remained stable across the five-week acclimation period in all treatment groups (**Table 2**).

Standard Length

Our best-fit model of *T. pennellii* standard length (mm) was a linear mixed-effects model with additive fixed effects of warming, pCO_2, and acclimation week. None of these predictors had a significant effect on length and mean length of T. pennellii hovered around 41 mm for the duration of the acclimation period in every treatment group (**Table 2**).

Fulton's Condition Factor

Our best-fit model of Fulton's condition factor (CF) in *T. pennellii* was a linear mixed-effects model with fixed effects of warming, pCO_2, and acclimation week. There was no significant effect of warming, pCO_2, or acclimation time on *T. pennellii* CF (**Table 2**).

Novel Object Test

Mean Velocity

Our best fit model of mean velocity (cm s^{-1}) in *T. pennellii* was a linear mixed-effects model with a two-way interaction between novelty and warming and a two-way interaction between week and warming. pCO_2 was included as a fixed effect, but did not show a significant main or interactive effect on *T. pennellii* velocity ($p < 0.58$, LRT). *T. pennellii* reduced their velocity in response to novelty in both temperature treatments, but this effect was greater under warming (–1°C est. = 0.93 cm s^{-1}, $t(105) = 4.65$, $p < 0.0001$; +2°C est. = 2.09 cm s^{-1}, $t(105) = 9.27$, $p < 0.0001$; NO×Temp est. = –1.16, $t(105) = –3.86$, $p = 0.0002$). *T. pennellii* exposed to +2°C reduced their velocity between weeks 2 and 5 (est. = 1.31 cm s^{-1}, $t(92.2) = 4.13$, $p = 0.0001$), but not under –1°C (est. = 0.28 cm s^{-1}, $t(92.0) = 0.88$, $p = 0.38$). Although the four-way interaction among novelty, time, temperature, and pCO_2 was not significant in our model ($p = 0.09$, LRT), the pronounced decline in velocity in the high pCO_2× +2°C treatment group suggests a potential complex interaction that we were not able to capture in the present study.

Total Time in Center Zone

Our best-fit model of total time *T. pennellii* spent in the center zone (s) was a Tweedie (log-link), with a three-way interaction among novelty, warming, and week. pCO_2 was included as a fixed effect and showed a weak positive trend (est. ratio = 1.29, $z = 1.65$, $p = 0.10$), but was not a significant predictor on the time *T. pennellii* spent in the center zone ($p = 0.10$, LRT). Warming alone had a pronounced effect: fish in the +2°C treatment group spent >2x as long in the center zone than those in –1°C treatment groups (est. ratio = 2.60, $z = 3.44$, $p < 0.001$). Warming had a strong effect on both the pre- and post- phases of the NOT over acclimation time; during the pre- phase of the NOTs at week 5, *T. pennellii* exposed to +2°C spent around 2.5x more time in the center zone than the –1°C group (est. ratio = 0.38, $z = –3.44$, $p < 0.001$). This indicates that warming elevated baseline exploratory behavior, independent of neophilia.

T. pennellii showed a strong response to the novel object that was markedly intensified by warming (est. ratio = 3.13, $z = 3.57$, $p < 0.001$). At week 2, after the novel object was introduced, *T. pennellii* increased their time in the center zone by >3x in both temperature treatments (–1°C est. ratio = 0.29, $z = –4.95$, $p < 0.0001$; +2°C est. ratio = 0.26, $z = –6.19$, $p < 0.0001$). By week 5, fish in the warming treatment spent nearly 4x the amount of time in the center zone after the novel object was added, whereas fish in the –1°C treatment group no longer showed a change (–1°C est. ratio = 0.79, z = -0.96, $p = 0.34$; +2°C est. ratio = 0.25, z = -6.83, p < 0.0001). Across weeks 2 and 5, *T. pennellii* in the +2°C treatment group sensitized to warming, but the –1°C treatment group habituated (–1°C est. ratio = 2.05, $z = 2.56$, $p = 0.01$; +2°C est. ratio = 0.52, $z = –2.72$, $p = 0.007$). Importantly, week, warming, and novelty did not interact additively over time. At week 2, *T. pennellii* in the +2°C treatment group spent ~64% less time in

the center zone than would be expected from additive effects of novelty and warming (est. ratio = 0.36, z = -2.21, p = 0.03). By week 5, this synergistic effect not only persisted but amplified; after the novel object was added, *T. pennellii* spent >8x longer in the center zone compared to the -1°C group (est. ratio = 8.13, z = 8.05, p < 0.0001).

Discussion

The Southern Ocean is among the world's coldest, most stenothermal marine environments, and accordingly Antarctic species are thought to be uniquely vulnerable to rapid global change (Gutt *et al.*, 2021; Peck *et al*., 2014; Rintoul *et al*., 2018). Species are responding differently to climate change, and understanding species-specific capacity to cope with variation in multiple co-occurring environmental conditions that have the potential to ask as stressors is critical for predicting how communities and ecosystems may respond to amplifying global climate change (Boyd *et al*., 2018; Breitburg *et al.*, 2015; Crain et al., 2008; Darling and Côté, 2008; Hoffman and Todgham, 2009; Todgham and Stillman, 2013). In this study, we examined how *Trematomus bernacchii* and *Trematomus pennellii*, two ecologically important species in McMurdo Sound (Barrera-Oro, 2002; La Mesa *et al*., 2004; McMullin *et al.*, 2017), may behaviorally respond to predicted ocean warming and ocean acidification.

Warming drives risky behavior

Broadly speaking, warming alone was the primary driver of the behavioral changes observed in both *T. bernacchii* and *T. pennellii*. Importantly, warming-induced alterations in behavior were dynamic and unpredictable, and dramatically amplified over the course

of the experiments in both species. Our results provide strong support for both reduced exploratory behavior and amplified neophilia in response to warming in both species; *T. bernacchii* and *T. pennellii* acclimated to +2°C reduced their mean velocity (i.e., swam less) and increased their time spent in the center zone in response to the novel object. This change in behavior was most apparent at the fifth week in both species, demonstrating an important synergism with time. Critically, though, we found important differences in the magnitude of response, where *T. pennellii* demonstrated the largest effect sizes in both behavioral metrics; in response to the novel object, *T. bernacchii* and *T. pennellii* acclimated to +2°C reduced their velocity by an estimated 1.35 and 2.09 cm s^{-1} and increased their total time spent in the center by ~3x and ~8x, respectively.

While previous work on Antarctic fish behavior is extremely limited, our results align with what has been previously described. A prior study from our group examining the interactive effects of warming and acidification on juvenile *T. bernacchii* also found that warming – not acidification – drove reductions in exploratory activity and increased anxiety, as assessed with open arena and scototaxis trials, respectively (Davis *et al*., 2018). While this study reported no effect of warming (nor pCO_2) on time spent in the center zone with a novel object, it is critical to consider experimental designs when comparing results. The novel object described in Davis *et al*. (2018) was a black tube (4 x 5.5 cm) sealed at one end, into which fishes were placed and not released for 30 s. While this is an excellent design for assessing refuge emergence and boldness – and indeed this study found some evidence of warming and acidification altering *T. bernacchii* refuge emergence – this design is not quite adequate for interpreting novel object recognition or neophilia. Novel objects must be carefully selected and should be

proportional to fish body size, brightly colored with complex shapes and – most importantly – animals must be allowed to choose to voluntarily interact with the object (Conrad *et al*., 2011; Hamilton, 2018; Roy *et al*., 2019; White *et al*., 2013). The initial starting placement of the fish inside the tube in Davis *et al.* (2018) is inherently forcing the fish to interact with the object and therefore precludes an accurate interpretation of how the fish voluntarily responds to a novel object. Differing experimental design has been an obstacle in behavioral research, and great efforts are being made to standardize experimental design where feasible (Näslund *et al*., 2015; Réale *et al*., 2007; Takola *et al*., 2021; White *et al*., 2013).

Subtle sensitivities to acidification

Many studies have shown that elevated pCO_2 can alter important behaviors in fishes, including boldness, anxiety, exploratory behavior, anti-predator responses, and feeding behavior (Alter *et al*., 2024; Cattano *et al*., 2018; Ishimatsu *et al*., 2008). But the relative importance and effect sizes of pCO_2 as a primary or interactive driver of behavioral modifications in fishes remains contentious (Alter *et al*., 2024; Browman, 2016, 2017; Clark *et al*., 2020a, 2020b; Clements *et al*., 2022; Esbaugh *et al*., 2023; Munday *et al*., 2020). In Antarctic fishes, previous research on the behavioral responses to pCO_2 is limited to one study (Davis *et al*., 2018), but there are a handful more studies examining the physiological effects of pCO_2 (Davis *et al*., 2016; Davis *et al*., 2018; Enzor *et al*., 2013; Enzor *et al*., 2017; Flynn *et al*., 2015; Hancock *et al*., 2020; Naslund *et al*., 2021; Strobel *et al*., 2012; Strobel *et al*., 2013). Five of these studies focused on, or included, *T. bernacchii* (Davis *et al*., 2016; Davis *et al*., 2018; Enzor *et al*., 2013; Enzor *et al*.,

2017; Naslund *et al*., 2021); none included *T. pennellii*. As such, this study presents the first experimental research on the concurrent effects of warming and acidification on *T. pennellii* at any life stage. Sensitivity to ocean acidification depends on life stage and habitat and it is thought that very young life stages (i.e., eggs and larvae) are particularly vulnerable, whereas juvenile life stages are generally rather robust (Davis *et al*., 2016; Flynn *et al*., 2015; Flynn and Todgham, 2018).

Contrary to our *a priori* predictions, we found only subtle impacts of acidification on behavior and growth in *T. bernacchii*, and no significant effects in *T. pennellii*. In *T. bernacchii*, moderate pCO_2 – but not high pCO_2 – drove a small but significant weight gain and an associated modest increase in condition factor (CF). Similarly, elevated pCO_2 briefly reduced exploratory swimming activity in *T. bernacchii*, but this effect was no longer seen after week 1. While elevated pCO_2 had no significant effect on neophilia in *T. bernacchii* at the $p < 0.05$ level, there was a modest indication of a three-way interaction between week, temperature, and pCO_2 (LRT, $p = 0.086$) in our model selection. This is most apparent in the -1°C treatment at 2 weeks, and in the +2°C treatment at 5 weeks (**Figure 2**). In both cases, the fish in the high pCO_2 treatments spent less time in the center zone compared to ambient and moderate pCO_2.

While not statistically significant, the subtle interaction among warming, acidification, and week in *T. bernacchii* suggests complex, non-additive behavioral responses to environmental conditions in these young Antarctic fishes. It is plausible that we were simply unable to resolve this relationship with the limited sample sizes in our study, especially considering the generally high variation observed in behavioral traits (Bell *et al.*, 2009; Biro and Stamps, 2008; Réale et al., 2007; Stamps and

Groothius, 2010; Wong and Candolin, 2015). Because of the unique sea ice phenology and boom/bust seasonal productivity cycles, Antarctic fishes may have evolved under larger fluctuations in pCO_2 than more temperate species, potentially promoting evolutionary adaptation to pCO_2 variability and a larger window of pCO_2 tolerance (Kapsenberg *et al*., 2015; Jones *et al*., 2023; Roden *et al*., 2016). We do not interpret our results as motivation for abandoning studying the effects of acidification on Antarctic fishes; rather, this work provides further evidence for subtle interactions between warming and acidification that could have important consequences at other life stages (particularly eggs and larvae), longer time scales, higher acidification and warming exposure levels, in different species, and/or when combined with other environmental conditions. Our results highlight a critical need for further research that may be able to better resolve these interacting potential stressors.

Synergism over time

While we did not see significant interactions of warming and acidification, we documented synergy (up to 8x) in these fishes between warming and time. In previous work, the acclimation period to warming and/or acidification stressors on early life stages (i.e., eggs, larvae, and juveniles) of Antarctic fishes has been limited to four weeks (Davis *et al*., 2016) and are typically closer to two or three weeks (Davis *et al*., 2018; Flynn *et al*., 2015; Flynn and Todgham, 2018), or have no acclimation period at all (Corso *et al*., 2024). In contrast, studies on adult life stages have spanned anywhere from a few days or weeks up to just under 10 weeks (summarized in Todgham and Mandic, 2020). The five-week acclimation period in this study extends the longest

experimental acclimation period on early life stages of any Antarctic fish by about a week, or 25%. Our finding of behavioral sensitization to novelty over time in both species emphasizes the critical need for longer-term studies to better understand how species may compensate – or sensitize – to changes in environmental conditions given enough time. The synergism of stressors with time is particularly important to consider given the dramatic phenological shifts and phenological mismatches facing polar ecosystems (Conroy *et al.*, 2023; Hague and Vichi, 2018; Kerby and Post, 2013; Post, 2017; Søreide *et al.*, 2010; Tedesco *et al.*, 2019). Antarctic research is limited by many logistical challenges and achieving longer-term studies will require creativity and collaboration between researchers and Antarctic research stations.

Assessing species-specific vulnerability to environmental stressors

The fitness implications associated with warming-induced neophilia and a freezing response are dependent on larger ecosystem dynamics; we simply do not yet have enough information to accurately assess if the observed behavioral responses are advantageous, deleterious, or neutral. On one hand, neophilia is thought to give a competitive advantage, as it is positively correlated with higher exploratory activity, adaptability, and innovation, which can enhance foraging efficiency and securing habitat (Wright *et al.*, 2010). On the other hand, neophilia could increase predation risk, especially if behavioral changes are mechanistically linked to interfered sensory processing of the environment (i.e., detecting food and predators). It is also possible that the costs and benefits associated with stronger neophilia may balance each other out.

Of the (limited) literature – primarily in experimental stress physiology – that empirically compares various aspects of stress tolerances of *T. bernacchii* and *T. pennellii*, there is a growing body of evidence that *T. pennellii* may have higher capacity than *T. bernacchii* to cope with predicted climate change stressors. On the organismal level, we previously found that non-laboratory acclimated juvenile *T. pennellii* had significantly higher body condition (as assessed by Fulton's condition factor, CF) compared to *T. bernacchii* (Naslund *et al.*, in review). In Chapter 2, however, we did not see a significant difference in CF between the two species. Our best explanation for this discrepancy is the collection year – *T. bernacchii* in Chapter 2 and (Naslund *et al*., in review) were collected in 2018 and 2019, respectively, whereas all *T. pennellii* were collected in 2019. Higher condition is generally assumed to be positively associated with higher performance, due to advantages like higher energy stores (Chellappa *et al*., 1995) and higher survival under thermal stress (Robinson *et al*., 2008). The intraspecific differences in CF across years may be evidence of interannual variability in intraspecific performance of these mixed species assemblages. A recent study on fishes found on the WAP found that adult *T. bernacchii* had poor condition relative to five other species (Kim *et al*., 2023). We also found lower median and substantial interspecific variability in CF of *T. bernacchii* in **Chapter 2**. Taken together with the present study, we interpret the available data to indicate that *T. pennellii* may generally, and/or more reliably, have higher condition than *T. bernacchii*.

Previous studies comparing the stress physiology of *T. bernacchii* and *T. pennellii* repeatedly point to *T. pennellii* having higher thermal tolerance than *T. bernacchii*. For example, adult *T. pennellii* survived significantly longer under acute, extreme heat

exposure of 14°C than *T. bernacchii*, both with and without a previous warming acclimation to 4°C (Podrabsky and Somero, 2006). In a cellular energy budget study, *T. pennellii* hepatocytes (liver cells) upregulated cellular respiration at high temperatures without a change in energy budget, whereas *T. bernacchii* hepatocytes showed limited metabolic capacity under the same conditions (Mark *et al*., 2005). Recent findings from our group indicate that *T. pennellii* may have higher mitochondrial respiration capacity than *T. bernacchii* at both adult and juvenile life stages (Mandic *et al.*, 2022). In our temperature preference study, juvenile *T. pennellii* preferred significantly warmer (+1°C) water compared to *T. bernacchii* (0°C) (Naslund *et al.*, in review). Notably, *T. bernacchii* often chose the coldest temperature available (-1.4°C), indicating they may prefer even colder temperatures if given the opportunity.

Ecological insights further support the idea that *T. pennellii* may have a stronger ability to cope with future oceans. Dietary analyses indicate *T. pennellii* have a more generalist diet compared to *T. bernacchii*; a more generalist diet may be adaptive under shifting food web conditions (McMullin, 2017). In another study, *T. pennellii* were highly abundant in habitats disturbed by iceberg scouring, suggesting they may be better able to recover from rapid habitat disturbances (Brenner, 2001). Taken together, available comparative data consistently suggest that *T. pennellii* could outperform *T. bernacchii* under predicted future ocean conditions.

Insight into juvenile growth trajectories

Understanding species-specific growth rates is an important component of assessing vulnerability to environmental stressors. Neither warming nor acidification affected

growth in either species over the five-week acclimation period. A previous study demonstrated that acclimation to +4°C dropped the growth rate and CF of adult *T. bernacchii* (Enzor *et al*., 2017). It is possible that any morphological impacts may be too subtle or too slow to detect within our five-week acclimation time, especially considering the long life spans and slow growth trajectories of these fishes (Mintenbeck *et al*., 2012; Todgham and Mandic, 2020).

Contrary to expectations, *T. pennellii* showed essentially no growth during the five-week acclimation period, whereas *T. bernacchii* grew modestly, consistent with expectations for laboratory acclimation and ample food. This snapshot of their differing juvenile growth trajectories demonstrates that – in addition to being smaller than *T. bernacchii* at both juvenile and adult life stages (**Chapter 2**, Artigues *et al*., 2003; Dewitt *et al.*, 1990) – *T. pennellii* may also grow more slowly. Generally, faster growth rates are thought to be adaptive, as larger individuals often have reduced predation risk, a competitive advantage for securing resources (i.e., food and habitat), and a higher capacity to tolerate environmental stressors (Anderson, 1988; Sogard, 1997; Ware, 1975). Documenting growth rates across juveniles for longer periods of time would be helpful to understand how different growth rates might be between these two species.

Larger body size and faster growth are not always “better”, though. Slower-growing individuals have lower energy demands, which is important in habitats with unpredictable resources, such as the Antarctic (Brown *et al*., 2004; Krause *et al*., 2000; Peck, 2016; Peck, 2018; Peck *et al.*, 2006; Pörtner *et al*., 2007; Urban, 2007). Polar species across taxa demonstrate very slow growth rates and delayed maturation, aligning with a slow pace-of-life strategy, and it is thought that this is essentially required

for life in such cold environments (Clarke, 1979; Peck, 2018; Rosa and Seibel, 2010). Smaller size can also reduce interspecific competition and enhance coexistence via resource partitioning (Schoener, 1974), and this has been described across fishes in more temperate systems (Dukowska and Grzybkowska, 2014; Franken and Hik, 2004; Svanbäck and Eklöv, 2004). Furthermore, under the size refuge hypothesis, smaller fish have access to microhabitats unavailable to larger individuals, which is thought to help them avoid detection and predation (Urban, 2007). A smaller body size could be extremely advantageous in their ice-reef habitat (**Chapter 4**), where they are consistently observed to hide in the reef microstructure. Differences in growth rates during juvenile stages can canalize ecological roles as fishes mature (Baras and Jobling, 2002), so more insight into how these mixed-species fish assemblages coexist in the same habitat is important to better understand their sensitivities to global climate change.

Ecosystem implications and future directions

We found that the two closely related species *T. bernacchii* and *T. pennellii* displayed similar risk-taking and exploratory behavior in response to OA and OW, indicating potentially conserved behavioral stress responses. *T. pennellii*, however, consistently exhibited substantially larger effect sizes, including spending significantly more time in risky zones, especially under acclimation to +2C. It is currently unclear if a more risk-prone strategy is adaptive or maladaptive in this ecosystem (Réale *et al*., 2010; Smith and Blumstein, 2008). While enhanced neophilia and risk-taking could improve adaptability in foraging success and habitat-use, these behaviors could also elevate

predation risk. Predicting the net outcome of altered behavioral strategies in these species is complex, and requires deeper research into ecological dynamics – such as predator-prey dynamics, intra- and inter-specific competitive interactions, and habitat utilization – across ontogeny (Werner and Gilliam, 1984).

The behavioral responses to novelty observed in these fishes in this study also strongly align with the hypothesis of a conserved freezing response in response to stress in these species (proposed in **Chapter 2**). Freezing behavior is an energetically efficient strategy and would be especially well-suited for the sedentary lifestyles described in many Antarctic fishes at both juvenile and adult life stages (**Chapter 4**; Kellermann, 1996; Montgomery and Clements, 2000; Vacchii *et al.*, 2004). It is thought that energy conservation is particularly important in polar ecosystems, where simply surviving is so energetically costly (Cheng and Detrich, 2007; Maes *et al.*, 2006; Peck *et al*., 2006).

Habitat complexity has been shown to shape exploratory-avoidant behavior in fishes (Ahmad and Richardson, 2013). At the juvenile life stage, *T. bernacchii* and *T. pennellii* live in a highly complex, ice-reef habitat (discussed in **Chapter 4**), where a freezing-response could be a simple, energy-saving strategy to avoid predation. We have observed both *T. bernacchii* and *T. pennellii* seemingly 'burrowing' into ice-reef structure *in situ*, as well as in fresh brash ice in preliminary laboratory habitat preference trials (AJF, unpublished data, 2019). Based on *in situ* observations and exploratory fish haul data, we also posit that *T. bernacchii* and *T. pennellii* may select slightly different microhabitats within the ice reef, where there appears to be substantial heterogeneity (AJF, pers. obs., 2019). Similar to intertidal pools, we think there may be pockets of

water within the ice reef – maintained by differential water flow through the reef and bright austral summer sunlight – with dramatically different temperature and salinity. We think juvenile *T. pennellii* may select warmer pockets closer to the summer sunlight (AJF, pers. obs. and unpublished data, 2019). Future studies incorporating habitat complexity and composition are necessary to better understand the exploratory-avoidance strategies in Antarctic fishes.

Unfortunately, we have thus far failed to curb anthropogenic emissions sufficiently to stay below the 1.5°C and 2°C warming benchmarks set by the Paris Climate Accord (UNFCCC, 2015), and many ecological impacts of climate change are already surpassing projected 'worst-case' scenarios (IPCC AR6, 2021). This study, conducted in 2018 and 2019, represents an optimistic, conservative lower bound of projected warming; future work should investigate higher increments of warming (e.g., +3 to 5 °C) to fully capture the possible range of ocean conditions these fishes are facing. Our findings hint at subtle, but potentially significant interaction between warming and acidification that we may not have fully resolved due to limited sample sizes and/or acclimation times. Future studies should prioritize long-term, higher power, multi-stressor experimental designs to better resolve these interactions and their ecological consequences.

Concluding remarks

The aim of this study was to examine species-specific responses to two co-occurring environmental conditions threatening the Antarctic, ocean warming and ocean acidification. There are extremely few studies on the behavior of *Trematomus bernacchii*

and *Trematomus pennellii*, and only one study on the effects of warming and acidification on behavior in *Trematomus bernacchii* (Davis *et al.*, 2018). Almost all of our knowledge about Antarctic fish behavior is from limited field observations and, more recently, limited *in situ* footage (Cziko *et al.*, 2020; Fox, 2015; Purser *et al.*, 2022; Rack *et al.*, 2012; Schmidt, 2020). Ecological observations can give ground-breaking insights into life history strategies, potential adaptations, and community interactions. Laboratory experiments such as the present study complement ecological observations by empirically simulating future ocean stressors, helping predict how species might cope with ocean change. Laboratory studies can be especially valuable in revealing nuanced behavioral differences that are not feasible to measure *in situ*, particularly due to technological limitations at below-freezing temperatures (e.g., limited battery life). Nuanced behavioral differences, especially in response to stressors, can have important ecological implications (Dingemanse and Wolf, 2013; Mittelbach *et al.*, 2014; Schmitz and Trussell, 2016; Sih *et al.*, 2004; Wong and Candolin, 2015). The present study aligns with the idea that behavior acts as the “key first response” to rapid environmental changes (Sih *et al.*, 2012), and emphasizes the value of integrating behavioral approaches into the robust physiological framework that underpins our understanding of Antarctic fishes.

Ultimately, our hope is that this work helps inform predictions of shifting population and community dynamics in McMurdo Sound, Antarctica. Species-specific differences in vulnerability to ocean change could drastically shift the distribution and abundance of benthic fishes, particularly if *T. bernacchii*, the more abundant fish, is more vulnerable. As oceans rapidly change with amplifying climate change, ecosystems

are predicted to restructure, and some species will be “winners” and some will be “losers” (Fetzer *et al.*, 2015). If *T. bernacchii* abundance declines due to a weaker ability to cope with climate change than *T. pennellii*, *T. pennellii* could experience an ecological release, or niche expansion. While direct competition between *T. bernacchii* and *T. pennellii* has not been empirically tested, the overlapping diets and habitats of these species suggests that competition may be at play (La Mesa, 2004; McMullin *et al*., 2017). Benthic Antarctic fishes play an especially important stabilizing role in the Antarctic food web (Cherel *et al.*, 2011; De Angelis, 1975; Hureau, 1994; McMullin *et al*., 2017) and thus may amplify climate change related perturbations throughout the larger food web (Kortsch *et al*., 2015; Walther, 2010). Understanding the capacities of benthic notothenioids to cope with environmental change is therefore critical to predicting larger ecosystem responses.

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Tables

Table 1. Average (± SD) seawater chemistry for each treatment condition for *T. bernacchii* and *T. pennellii* experiments.

Treatment	Temperature (°C)	Salinity (ppt)	Total Alkalinity (μmol kg^{-1})	pH	pCO_2 (μatm)
T. bernacchii					
Incoming seawater	-1.29 ± 0.13	33.5 ± 0.1	2352 ± 2	7.97 ± 0.01	486 ± 9
Ambient pCO_2 x Ambient Temp	-0.74 ± 0.39	33.5 ± 0.1	2353 ± 2	7.98 ± 0.02	473 ± 18
Ambient pCO_2 x High Temp	2.60 ± 0.22	33.6 ± 0.1	2354 ± 3	7.94 ± 0.02	527 ± 30
Moderate pCO_2 x Ambient Temp	-0.70 ± 0.38	33.5 ± 0.1	2354 ± 3	7.78 ± 0.02	774 ± 31
Moderate pCO_2 x High Temp	2.60 ± 0.24	33.6 ± 0.1	2353 ± 3	7.77 ± 0.02	809 ± 30
High pCO_2 x Ambient Temp	-0.76 ± 0.39	33.6 ± 0.1	2353 ± 3	7.67 ± 0.03	1022 ± 66
High pCO_2 x High Temp	2.61 ± 0.25	33.6 ± 0.1	2353 ± 3	7.63 ± 0.02	1122 ± 53
T. pennellii					
Incoming seawater	-1.32 ± 0.06	33.4 ± 0.1	2353 ± 2	7.98 ± 0.01	468 ± 14
Ambient pCO_2 x Ambient Temp	-1.05 ± 0.08	33.1 ± 0.4	2354 ± 2	8.00 ± 0.01	446 ± 16
Ambient pCO_2 x High Temp	2.19 ± 0.13	33.3 ± 0.2	2355 ± 2	7.96 ± 0.03	498 ± 36
High pCO_2 x Ambient Temp	-1.04 ± 0.07	33.3 ± 0.3	2355 ± 3	7.68 ± 0.02	987 ± 51
High pCO_2 x High Temp	2.12 ± 0.13	33.3 ± 0.3	2354 ± 4	7.66 ± 0.01	1048 ± 36

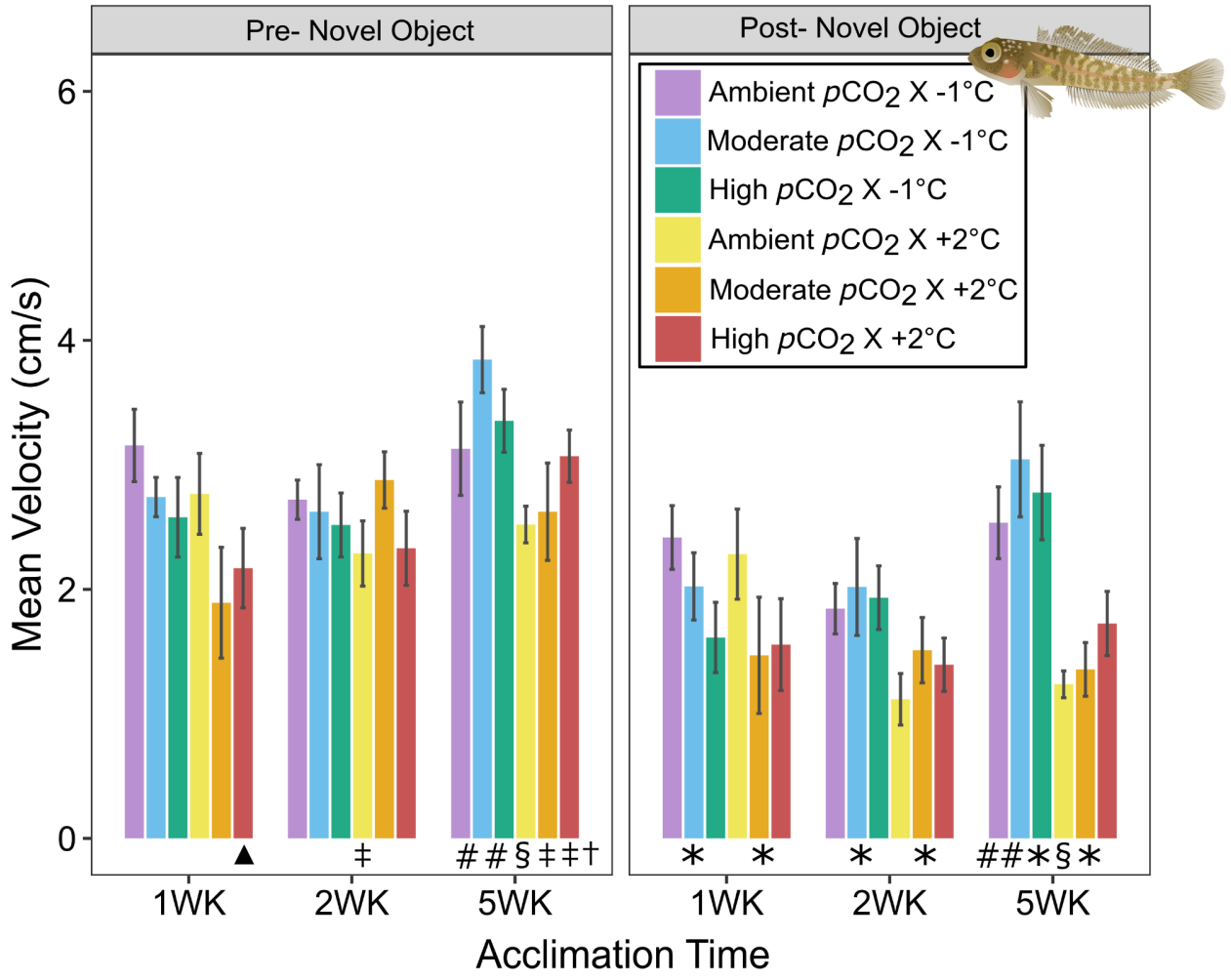

Figure 1. Mean swimming velocity (cm/s) of *Trematomus bernacchii* pre- novel object (left panel) and post- novel object (right panel) at acclimation time sampling point (1 week, 2 weeks, and 5 weeks). Data are presented as bar plots, with the bar and error bars representing the mean ± standard deviation, for each treatment: ambient *p*CO2 x -1°C (lavender), moderate pCO_2 x -1°C (aqua), high pCO_2 x -1°C (green), ambient pCO_2 x +2°C (yellow), moderate pCO_2 x +2°C (orange), high pCO_2 x +2°C (red). Significant differences in mean velocity (cm/s) between pre- and post- NO per temperature

treatment at acclimation week are denoted with * on the right panel. Significant differences between temperature treatments at acclimation week pre- and post- NO are designated with §. Significant differences between 1wk and 5wk and 2wk and 5wk of each temperature treatment pre- and post- NO are designated with #. Significant differences between 2wk and 5wk for each pCO_2 treatment are denoted with † and significant differences between 2wk and 5wk for each pCO_2 treatment are denoted with ‡ on the left panel. Significant differences of elevated pCO_2 treatments compared to ambient pCO_2 at each week are denoted with ▲. All estimates and test statistics can be found in **S. Table 1.**

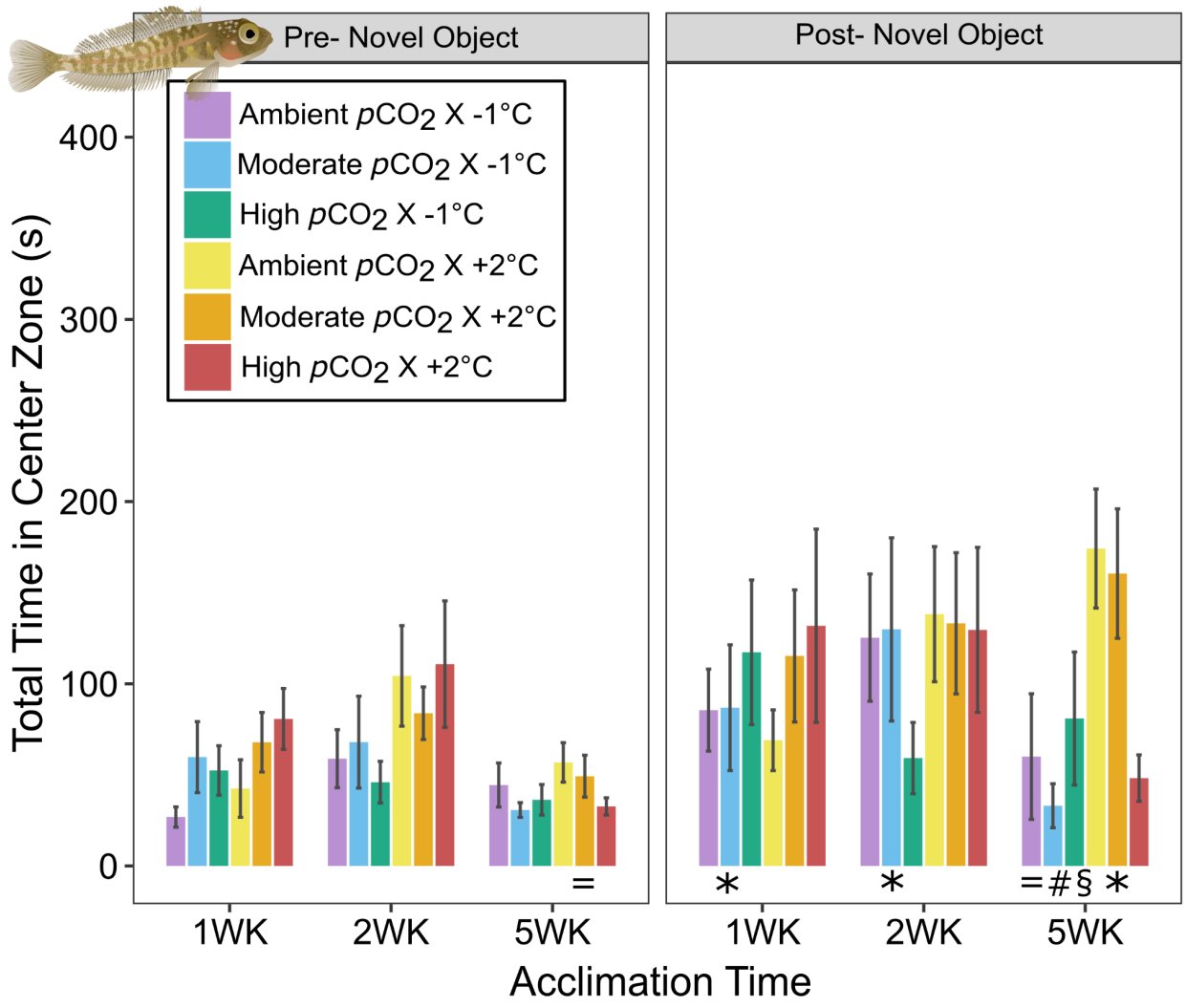

Figure 2. Total time spent in the center zone (s) of *Trematomus bernacchii* pre- novel object (left panel) and post- novel object (right panel) at acclimation time sampling point (1 week, 2 weeks, and 5 weeks). Data are presented as bar plots, with the bar and error bars representing the mean ± standard deviation, for each treatment: ambient *p*CO2 x -1°C (lavender), moderate pCO_2 x -1°C (aqua), high pCO_2 x -1°C (green), ambient pCO_2 x +2°C (yellow), moderate pCO_2 x +2°C (orange), high pCO_2 x +2°C (red). Significant differences in mean velocity (cm/s) between pre- and post- NO per temperature

treatment at acclimation week are denoted with * on the right panel. Significant differences between temperature treatments at acclimation week pre- and post- NO are denoted with §. Significant differences between 1wk and 5wk at each temperature treatment pre- and post- NO are denoted with # and significant differences between 2wk and 5wk at each temperature treatment pre- and post-NO are designated with =. All estimates and test statistics can be found in **S. Table 1.**

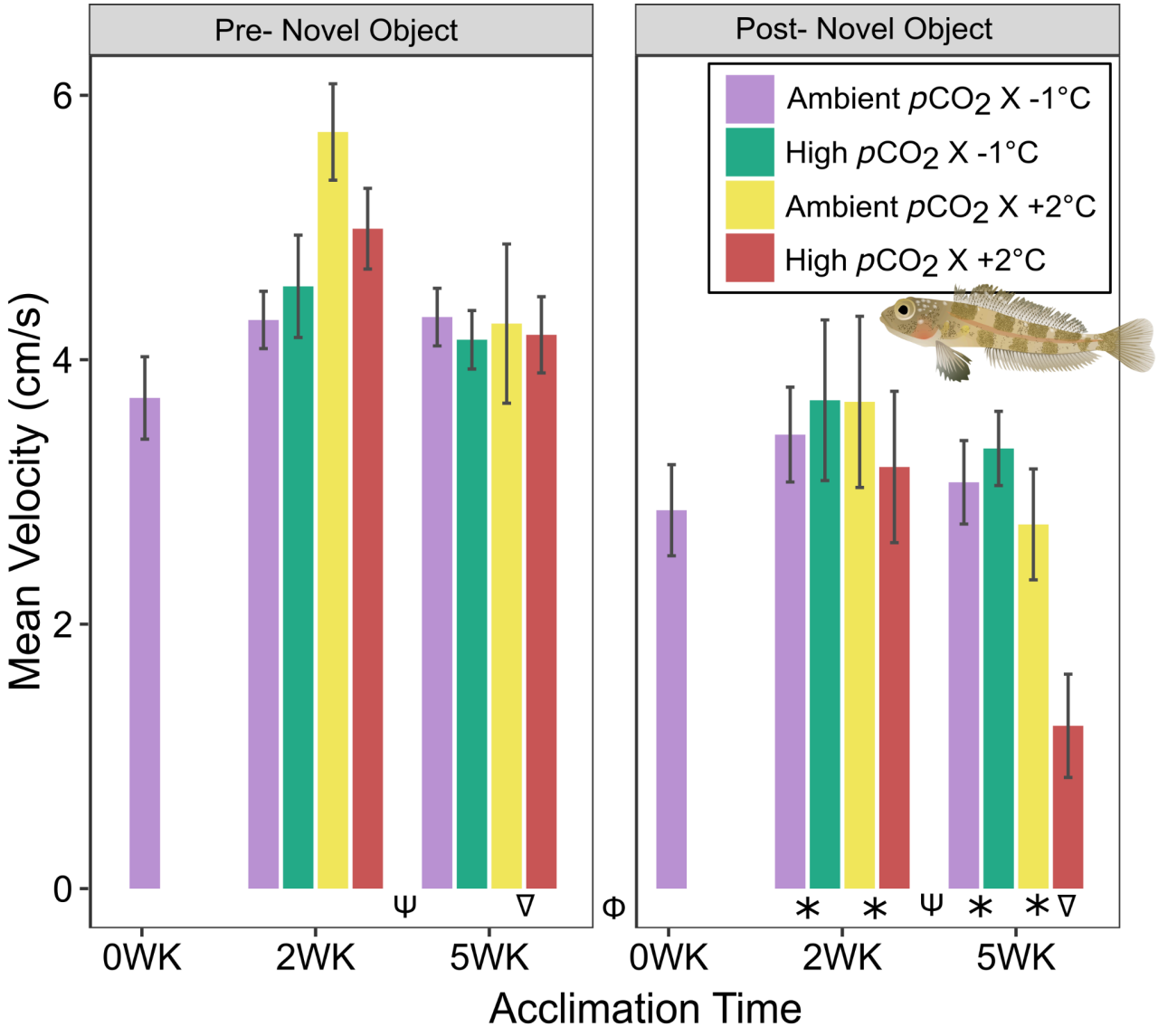

Figure 3. Mean swimming velocity (cm/s) of *Trematomus pennellii* pre- novel object (left panel) and post- novel object (right panel) at acclimation time sampling point (0 week, 2 weeks, and 5 weeks). Data are presented as bar plots, with the bar and error bars representing the mean ± standard deviation, for each treatment: ambient *p*CO2 x -1°C (lavender), high pCO_2 x -1°C (green), ambient pCO_2 x +2°C (yellow), high pCO_2 x +2°C (red). The significant difference between pre- and post-NO is denoted with Φ. The significant difference between 2wk and 5wk is denoted with Ψ in both panels.

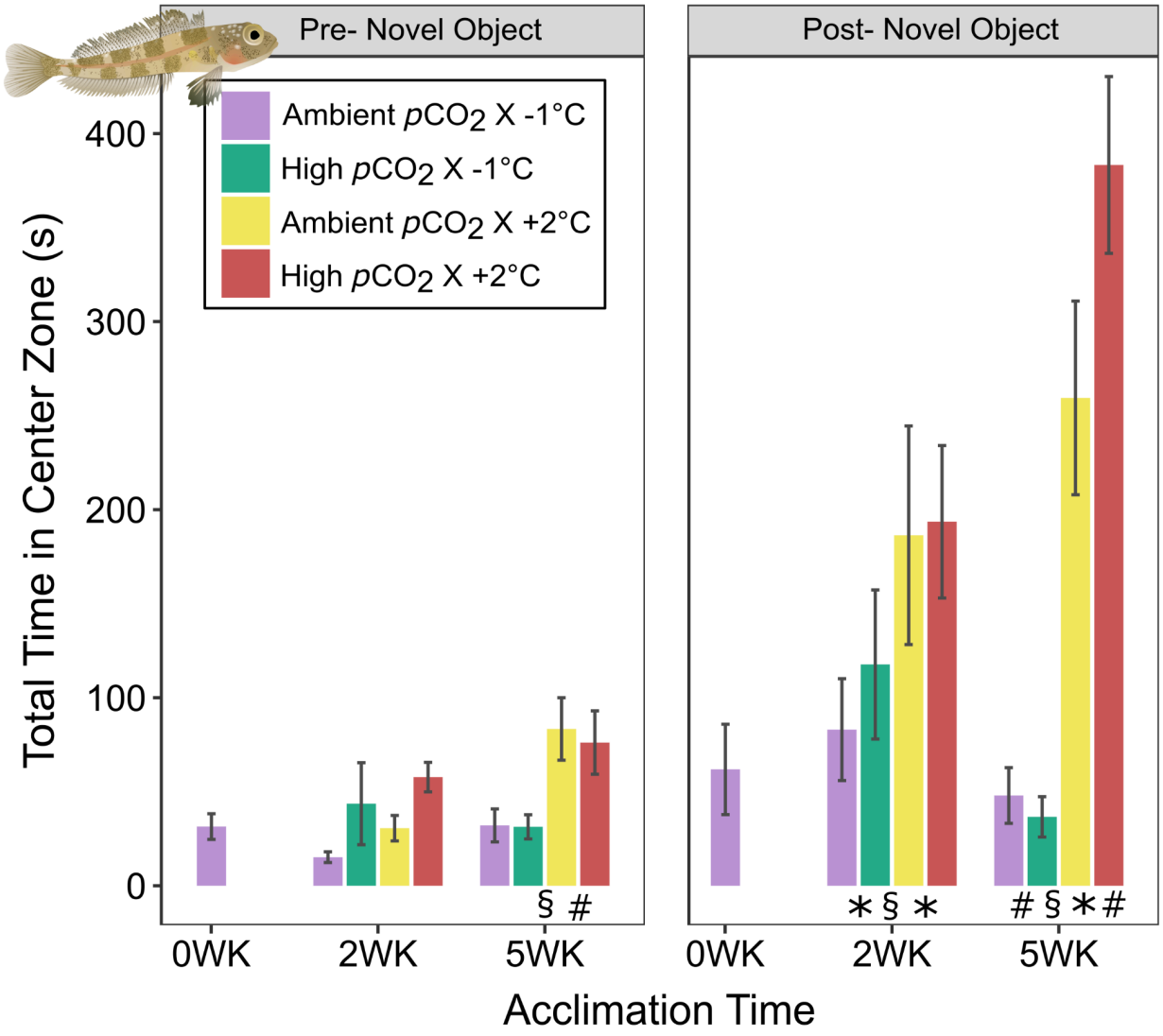

Figure 4. Total time spent in the center zone (s) of *Trematomus pennellii* pre- novel object (left panel) and post- novel object (right panel) at acclimation time sampling point (0 week, 2 weeks, and 5 weeks). Data are presented as bar plots, with the bar and error bars representing the mean ± standard deviation, for each treatment: ambient *p*CO2 x -1°C (lavender), high pCO_2 x -1°C (green), ambient pCO_2 x +2°C (yellow), high pCO_2 x +2°C (red). Significant differences between pre- and post- NO per temperature treatment at acclimation week are denoted with * on the right panel. Significant

differences between temperature treatments at acclimation week pre- and post- NO are denoted with §. Significant differences between 2wk and 5wk at each temperature treatment pre- and post- NO are denoted with #. All estimates and test statistics can be found in **S. Table 1.**

Appendix

S. Table 1. Adjusted contrasts from post-hoc significance testing for all models.

Reported p-values were adjusted using the Benjamini-Hochberg method.

NOVEL OBJECT - *T rematomus bernacchii*					
contrasts	**estimate**	**s.e.**	**df**	**t- ratio or z-ratio**	**adjusted p-value**
Mean Velocity (cm/s)					
1wk -1°C: pre – post NO	0.81	0.16	209	5.11	<0.001
2wk -1°C: pre – post NO	0.69	0.16	209	4.35	<0.001
5wk -1°C: pre – post NO	0.66	0.16	209	4.16	<0.001
1wk +2°C: pre – post NO	0.51	0.16	209	3.18	0.009
2wk +2°C: pre – post NO	1.14	0.16	219	7.05	<0.001
5wk +2°C: pre – post NO	1.30	0.16	209	8.21	<0.001
1wk pre NO: -1°C – +2°C	0.54	0.25	110	2.15	0.07
1wk post NO: -1°C – +2°C	0.24	0.25	110	0.97	0.48
2wk pre NO: -1°C – +2°C	0.12	0.25	109	0.48	0.74
2wk post NO: -1°C – +2°C	0.58	0.25	110	2.29	0.06
5wk pre NO: -1°C – +2°C	0.71	0.25	108	2.82	0.02
5wk post NO: -1°C – +2°C	1.35	0.25	108	5.37	<0.001
-1°C pre NO: 1wk – 2wk	0.21	0.24	290	0.86	0.50
-1°C pre NO: 1wk – 5wk	-0.62	0.24	290	-2.59	0.03
-1°C pre NO: 2wk – 5wk	-0.82	0.24	290	-3.45	0.004
+2°C pre NO: 1wk – 2wk	-0.22	0.24	291	-0.90	0.49
+2°C pre NO: 1wk – 5wk	-0.45	0.24	290	-1.89	0.13
+2°C pre NO: 2wk – 5wk	-0.24	0.24	291	-1.00	0.46
-1°C post NO: 1wk – 2wk	0.09	0.24	290	0.36	0.81
-1°C post NO: 1wk – 5wk	-0.77	0.24	290	-3.23	0.008

-1°C post NO: 2wk – 5wk	-0.85	0.24	290	-3.58	0.003
+2°C post NO: 1wk – 2wk	0.42	0.24	292	1.74	0.17
+2°C post NO: 1wk – 5wk	0.33	0.24	290	1.39	0.30
+2°C post NO: 2wk – 5wk	-0.09	0.24	291	-0.36	0.81
Ambient pCO_2: 1wk – 2wk	0.65	0.26	189	2.53	0.04
Ambient pCO_2: 1wk – 5wk	0.30	0.26	189	1.17	0.39
Ambient pCO_2: 2wk – 5wk	-0.35	0.26	189	-1.37	0.30
Moderate pCO_2: 1wk – 2wk	-0.22	0.26	189	-0.84	0.51
Moderate pCO_2: 1wk – 5wk	-0.68	0.26	189	-2.60	0.03
Moderate pCO_2: 2wk – 5wk	-0.46	0.26	189	-1.78	0.16
High pCO_2: 1wk – 2wk	-0.06	0.26	189	-0.25	0.87
High pCO_2: 1wk – 5wk	-0.75	0.26	189	-2.92	0.02
High pCO_2: 2wk – 5wk	-0.69	0.26	189	-2.67	0.03
1wk: Ambient pCO_2 – Moderate pCO_2	0.62	0.28	73	2.22	0.07
1wk: Ambient pCO_2 – High pCO_2	0.68	0.27	71	2.46	0.04
1wk: Moderate pCO_2 – High pCO_2	0.06	0.28	73	0.22	0.87
2wk: Ambient pCO_2 – Moderate pCO_2	-0.26	0.28	71	-0.93	0.49
2wk: Ambient pCO_2 – High pCO_2	-0.04	0.28	71	-0.15	0.91
2wk: Moderate pCO_2 – High pCO_2	0.21	0.27	71	0.78	0.53
5wk: Ambient pCO_2 – Moderate pCO_2	-0.36	0.27	71	-1.32	0.32
5wk: Ambient pCO_2 – High pCO_2	-0.38	0.27	71	-1.37	0.30
5wk: Moderate pCO_2 – High pCO_2	-0.01	0.27	71	-0.05	0.96
Total Time Spent in Center Zone (s)					
1wk -1°C: pre – post NO	0.51	0.11	Inf	-3.15	<0.001
2wk -1°C: pre – post NO	0.59	0.12	Inf	-2.51	0.035
5wk -1°C: pre – post NO	0.82	0.19	Inf	-0.82	0.51
1wk +2°C: pre – post NO	0.67	0.14	Inf	-1.93	0.12

2wk +2°C: pre – post NO	0.75	0.15	Inf	-1.48	0.29
5wk +2°C: pre – post NO	0.39	0.08	Inf	-4.61	<0.001
1wk pre NO: -1°C – +2°C	0.74	0.19	Inf	-1.18	0.38
1wk post NO: -1°C – +2°C	0.97	0.23	Inf	-0.13	0.91
2wk pre NO: -1°C – +2°C	0.60	0.15	Inf	-2.12	0.08
2wk post NO: -1°C – +2°C	0.76	0.18	Inf	-1.21	0.38
5wk pre NO: -1°C – +2°C	0.83	0.22	Inf	-0.73	0.56
5wk post NO: -1°C – +2°C	0.39	0.10	Inf	-3.79	0.002
-1°C pre NO: 1wk – 2wk	0.80	0.20	Inf	-0.89	0.75
-1°C pre NO: 1wk – 5wk	1.17	0.31	Inf	0.60	0.87
-1°C pre NO: 2wk – 5wk	1.47	0.38	Inf	1.50	0.43
+2°C pre NO: 1wk – 2wk	0.65	0.16	Inf	-1.80	0.30
+2°C pre NO: 1wk – 5wk	1.31	0.34	Inf	1.07	0.43
+2°C pre NO: 2wk – 5wk	2.04	0.50	Inf	2.89	0.02
-1°C post NO: 1wk – 2wk	0.93	0.22	Inf	-0.31	0.49
-1°C post NO: 1wk – 5wk	1.90	0.48	Inf	2.53	0.04
-1°C post NO: 2wk – 5wk	2.04	0.52	Inf	2.83	0.02
+2°C post NO: 1wk – 2wk	0.73	0.17	Inf	-1.36	0.30
+2°C post NO: 1wk – 5wk	0.76	0.18	Inf	-1.15	0.39
+2°C post NO: 2wk – 5wk	1.05	0.24	Inf	0.22	0.87
NOVEL OBJECT - *Trematomus pennellii*					
contrasts	**estimate**	**s.e.**	**df**	**t- ratio or z-ratio**	**adjusted p-value**
Mean Velocity (cm/s)					
pre – post NO	1.51	0.15	105	10.03	0.0002
2wk – 5wk	0.8	0.22	92	3.56	0.001
Ambient pCO_2 – High pCO_2	0.26	0.34	9	0.79	0.45
-1°C: pre – post NO	0.93	0.20	105	4.65	0.0003
+2°C: pre – post NO	2.09	0.23	105	9.27	0.0003

pre NO: -1°C – +2°C	-0.46	0.37	13	-1.26	0.29
post NO: -1°C – +2°C	0.70	0.37	13	1.90	0.12
-1°C: 2wk – 5wk	0.28	0.31	92	0.88	0.40
+2°C: 2wk – 5wk	1.31	0.32	92	4.13	0.0003
2wk: -1°C – +2°C	-0.4	0.4	18	-0.99	0.39
5wk: -1°C – +2°C	0.64	0.41	19	1.57	0.18
Total Time Spent in Center Zone (s)					
2wk -1°C: pre – post NO	0.29	0.07	Inf	-4.95	0.0003
5wk -1°C: pre – post NO	0.79	0.2	Inf	-0.96	0.39
2wk +2°C: pre – post NO	0.26	0.06	Inf	-6.19	0.0003
5wk +2°C: pre – post NO	0.25	0.05	Inf	-6.83	0.0003
2wk pre NO: -1°C – +2°C	0.55	0.16	Inf	-2.04	0.06
2wk post NO: -1°C – +2°C	0.48	0.13	Inf	-2.81	0.01
5wk pre NO: -1°C – +2°C	0.38	0.11	Inf	-3.44	0.001
5wk post NO: -1°C – +2°C	0.12	0.03	Inf	-8.05	0.0003
-1°C pre NO: 2wk – 5wk	0.76	0.23	Inf	-0.92	0.39
+2°C pre NO: 2wk – 5wk	0.53	0.14	Inf	-2.34	0.03
-1°C post NO: 2wk – 5wk	2.05	0.57	Inf	2.56	0.02
+2°C post NO: 2wk – 5wk	0.52	0.13	Inf	-2.72	0.01

CHAPTER 4

Ice reefs; and a new perspective on ice-association in polar fishes

Abstract

Sea ice is a central habitat in polar oceans that shaped physical and biological processes both locally and globally. While considerable research has focused on the important role of sea ice for charismatic megafauna, and more recently for planktonic communities, the role of sea ice for fishes remains underappreciated. This chapter focuses on ice-association in polar fishes, and introduces a novel framework for understanding sea ice habitat for young fishes: the ice reef. Abundant *in situ* observations document mixed species assemblages of notothenioids sitting in near-shore ice reefs – highly complex, three-dimensional ice structures formed by platelet ice, anchor ice, and ice brinicles. I look to other well-described, reef-like habitats found across temperate regions, such as coral reefs, kelp forests, and oyster beds, to emphasize the potentially critical function that ice reefs play for young polar fishes for shelter and feeding opportunities. I first examine sea ice dynamics in the Arctic and Antarctic and highlight how differences in geography, wind patterns, and freshwater inputs yield distinct ice formation, stability, and phenology in each region. I then propose five stages of ice reef emergence: Anchoring, Growth, Seeding, Recruitment, and Disintegration, to describe how abiotic and biotic processes together create structurally complex habitat and foster entire ecological communities. I synthesize the literature and *in situ* observations and show that a diversity of fish species exhibit repeated behavioral, physiological, and morphological adaptations that suggest an ice-associated or ice-obligate life history. I emphasize that ice-association is a likely widespread life

history strategy in polar fishes, rather than a unique adaptation only found in a select few species, and that ice reefs may be a critical nursery habitat for young polar fishes. Finally, I discuss the climate vulnerability of ice reefs: as polar warming accelerates and alters ice phenology and characteristics, the loss of ice reefs may imperil fish recruitment, reshape community stability, and erode ecosystem services such as nutrient and carbon cycling. By positioning ice reefs at the nexus of physical processes and biological adaptations, this chapter invites a cross-disciplinary perspective on polar marine ecosystems and underscores the urgent need to study ice reef extent, function, and resilience under rapid environmental change.

Introduction

Sea ice is a defining feature of polar ecosystems and has been a priority subject of research for decades (Dieckmann and Hellmer, 2010; Elias, 2021; Fogg, 1992; Weeks, 1998). Interdisciplinary attention on sea ice is only growing and the urgency of sea ice research cannot be overstated, as a) it is rapidly disappearing at a pace that far surpasses our ability to study it, and b) it remains understudied compared to more temperate and accessible systems. The Polar Regions have long been bellwethers for the impacts of the climate crisis and they are critical study systems across disciplines. Sea ice is an important driver of oceanographic and atmospheric processes, such as global ocean circulation, nutrient cycling, sea surface - atmosphere exchange, weather patterns, and albedo and oceanic heat uptake (Brandon *et al.*, 2010; Dieckmann and Hellmer, 2010). Sea ice is also of great interest to life scientists, as it provides an important and unique habitat for many species (Bluhm *et al*., 2010; Macias-Fauria and

Post, 2018; Tynan *et al*., 2010). Importantly, “ice is life” for many Indigenous peoples, including the Inuit, Chukchi and Yupik, and Unangax̂ (Elias, 2021; Watt-Cloutier, 2018).

While sea ice is characteristic of both the Arctic and Antarctic, sea ice dynamics vary markedly between the poles. The Arctic and Antarctic are fundamentally different because the Arctic is an ocean surrounded by land, whereas the Antarctic is land surrounded by ocean. This inherent difference drives distinct oceanographic processes that affect the seasonality, stability, and extent of sea ice (Brandon *et al*., 2010). Arctic sea ice is greatly influenced by the surrounding continents, for example from significant riverine input, which creates freshwater pycnoclines (see **Table 1. Glossary of Terms**) that can enhance sea ice formation by raising the freezing point of the surface water . The Arctic is expected to dramatically freshen (~1-1.5 psu by 2100) from rapid sea ice loss and concurrent amplifying riverine input, primarily due to increased precipitation and earlier snowmelt (Li and Fedorov, 2021; Shu *et al*., 2019). Freshening enhances stratification, altering the timing and thickness of sea ice formation, shoaling the nutricline and trapping nutrients in a thin layer of fresh, warm surface water, and creating a potential osmoregulatory stressor for marine osmoconformers (Li and Fedorov, 2021; Shu *et al*., 2019). Enhanced freshening and stratification of the Arctic is already weakening the Atlantic Meridional Overturning Circulation (AMOC) (Caesar *et al*., 2021, 2022; Kilbourne *et al.,* 2022) and threatens the global oceanic and atmospheric circulation patterns that regulate climate across the globe (Lenton *et al*., 2019). In contrast, the Antarctic does not have riverine input (apart from glacial melting) and, therefore much less freshening, so generally the Antarctic experiences greater vertical mixing than the Arctic (Rabe *et al*., 2023).

In the Antarctic, sea ice formation is primarily influenced by strong katabatic winds and the Antarctic Circumpolar Current; together, these forces keep the ice relatively stable and promote landfast ice, resulting in large expanses of continuous ice (Bennetts *et al*., 2024; Fraser *et al*., 2023). In the Arctic, however, winds and ocean currents are more chaotic and flow in contrasting directions (Timmermans and Marshall, 2020). The Beaufort Gyre and transpolar drift also push the sea ice away from the center, where it then is constrained by the surrounding continents. With limited landfast ice, the sea ice moves much more and ice floes are constantly colliding and fragmenting. While the sea ice in both poles experiences seasonal fluctuations in thickness and extent, the seasonality of Arctic sea ice is much more pronounced than that of Antarctic sea ice. Antarctica's extensive continental shelves promote sea ice stability, as well as the formation of platelet ice and pelagic ice nucleation (i.e., frazil ice) (Hoppmann *et al.*, 2020; Langhorne *et al*., 2015; Petrich and Eicken, 2010; Tsang and Hanley, 1985).

One unifying feature of the poles is the extreme seasonality in photoperiod, which seasonally regulates a tightly coupled sequence of ecological interactions across trophic levels (Brandon *et al*., 2010; Clarke, 1988). Sea ice grows, both in thickness and extent, through the winter months and seasonally thins and breaks out in the summer from the warm 24-hour midnight sun. Sea ice attenuates light penetration and drives nutrient fluxes, which in turn regulate the timing and intensity of primary production and the cascading dynamics of marine food webs (Arrigo *et al*., 2010; Massom and Stammerjohn, 2010). As a result of these seasonal synchronies, both the Arctic and Antarctic are characterized by pronounced seasonal pulses of nutrients followed by

extended food-limited periods (Arrigo *et al.*, 2010). This phenological cycle structures the availability of resources across trophic levels and constrains the life history timing of ice-associated species.

Sea ice is extremely important for organisms that live in the poles and has long been an important parameter to designate ecological zones. At the foundation of the food web, sea ice provides an important habitat for phytoplankton and other microorganisms, as well as extensive substrate, close to the light, where algae seasonally proliferates (Arrigo *et al*., 2010; Bluhm *et al.*, 2010; Tynan *et al.*, 2010). Platelet ice, in particular, provides expansive surface area for algal growth (Mangoni *et al.*, 2009) and rapid nutrient exchange (Arrigo and Thomas, 2004; Thomas *et al*., 2010). Phytoplankton blooms are closely tied to seasonal ice thinning, which promotes light penetration to subsurface sea ice, and sea ice break-out – as ice melts it releases nutrients which, paired with solar photic and thermal energy input, triggers seasonal phytoplankton blooms. Copepods (in the Arctic) and krill and amphipods (in the Antarctic) rely on the sea ice for energy and nutrients and are critical links between lower- and higher- levels of the food web (Barrera-Oro, 2002). Sea ice is also critical habitat for breeding, feeding, and shelter for species across trophic levels (for a review, see Tynan *et al*., 2010), particularly sea-birds and marine mammals. Finally, the Arctic sea ice has been life-giving to Indigenous peoples for millennia, whereas there are no Indigenous peoples that rely on Antarctic sea ice (it is important to note, however, that there is some evidence of Maori exploration of the Southern Ocean) (Wehi *et al.*, 2021a, 2021b).

Attention has recently grown on the role that sea ice plays for fishes, especially after the recent notable discovery of sea ice providing nursery habitat for silverfish eggs (Bottaro *et al*., 2009; Moline *et al*., 2008; Vacchi *et al*., 2012). Only a handful of polar fishes have been described as ice-associated, and generally an ice-associative strategy has been regarded as an interesting, unique exception, rather than the norm, for polar fishes. We propose that an ice-associative strategy may be far more prevalent and ecologically important than has been previously characterized. It is easy for humans, as terrestrial mammals, to see the obvious role that ice plays for the charismatic birds and mammals that catch our attention. Few humans have had the opportunity to observe polar fishes in a similar manner; we emphasize that when given the opportunity, it is just as clear that sea ice is critical habitat for polar fishes.

Ice Reefs

The near-shore Antarctic environment is characterized by otherworldly, dynamic ice formations that grow and shift on rapid temporal scales (hours to days) throughout the austral summer season (AJF, pers. obs., 2019). Common microscale ice features include platelet ice, anchor ice, and ice brinicles (**Figure 1**). Platelet ice forms when freshwater underneath the ice shelf moves to shallower depths, raising the pressure-dependent freezing point, and supercooling *in situ* to form small ice crystals (Langhorne *et al*., 2015; Buffo *et al*., 2017). Anchor ice forms attached to the benthos in depths shallower than ~5m along the shoreline. Ice brinicles, which descend down from the surface sea ice, form due to saltwater extrusion as seawater freezes (Cartwright *et al.*, 2013). All of these ice structures continue to grow if the surrounding water is cool enough (Langhorne *et al*., 2015), and even grow into large underwater ice caves along

the shoreline (large enough for adult Weddell seals to swim into), with platelet ice growing from every direction (**Figure 1**). Together, these ice structures form reef-like habitats, which we henceforth refer to as ice reefs.

Platelet ice appears to be the critical building block of ice reefs. Platelet ice was long considered a uniquely Antarctic phenomenon, coupled with ice shelf outflows (Hoppmann *et al*., 2020). But sub-ice platelet ice layers were recently reported from MOSAiC – the Multidisciplinary drifting Observatory for the Study of Arctic Climate – confirming what had previously been only sporadically documented and largely overlooked: platelet ice is also found in the Arctic (Jeffries *et al*., 1995; Katlein *et al*., 2020). Using remotely operated vehicles (ROVs), researchers documented widespread, complex, multi-dimensional platelet ice structures during the boreal winter beneath all surveyed ice types, including level ice, snow-covered floes, pressure ridges, and first-year ice (Katlein *et al*., 2020). Their published photography and videography are strikingly similar to platelet structures observed in McMurdo Sound ice reefs. This finding completely reshapes our current understanding of the geographic range of potential sea ice habitat and encourages new cross-polar perspectives on sea ice habitat ecosystems. The ecological significance of Arctic platelet ice layers remains unknown. Amphipods (thought to be *Apherusa glacialis*) were the only ice-associated biota reported in the platelet ice matrix, but – notably – their observed “maneuvering through the maze of crystal blades” is reminiscent of amphipod activity commonly seen in McMurdo platelet ice layers (AJF, pers. obs., 2019; Hoppmann *et al.*, 2020; Moline *et al*., 2008) – suggesting functional interaction with the structure, even in the full polar night. No observations of fish have yet been reported. This behavioral parallel raises

important phenological questions about whether Arctic platelet ice structure persists into the summer months and whether it supports seasonal ice-associated communities akin to those found in the Antarctic ice reefs.

Sea ice is often described as a canopy, ceiling, cover, or platform, but we propose a novel perspective: the ice reef framework, highlighting its ecological function. We propose the word ‘reef’ intentionally, because we posit that sea ice structures play a role comparable to that of structured habitats in more temperate systems, such as coral reefs, eelgrass meadows, kelp forests, mangrove roots, oyster reefs, rock reefs, and sargassum rafts (**Table 2**). In these structured habitats, abiotic and biotic components together create three-dimensional habitat complexity that act as skeletons – they scaffold attachment sites, shelter, and stability and promote the rich biodiversity that these habitats foster (**Table 2**). For example, coral polyps require a hard substrate to attach and grow, and as the coral grows, the calcium carbonate skeleton solidifies and becomes the physical reef structure. Similarly, sea ice microstructure can be thought of as the skeleton of the sea ice reef; it provides a complex structure where micro and macrofauna thrive. Sea ice is generally not thought of as biotic in most disciplines – and in its true sense, ice itself certainly is abiotic – but by shifting our framing to view sea ice as living, the role it plays becomes clear. Sea ice has distinctly biotic characteristics: it is a highly dynamic substrate that grows and changes over time, it provides habitat, and it supplies nutrients to the organisms that grow and live in, on, and under the ice. Understanding sea ice habitat with a ‘reef’ framework captures the important role that sea ice plays in the ecosystem; the ice itself is integral.

An essential element to the reef framework is that it also emphasizes that sea ice cover is tightly coupled with the benthos. The interdependence of the benthic, pelagic, and cryopelagic zones is relatively well established in terms of energy and nutrient transfer (Arrigo *et al*., 2010; Cautin *et al*., 2024; Grebmeier and Barry, 1991; McMullin *et al*., 2017; Smith *et al*., 2012), yet they are still often considered as separate zones – particularly with respect to habitat, and in physiological research. Foundational Antarctic physiological studies, including those focused on fishes, typically associated species with one zone (Eastman, 1991). Importantly, these studies relied on adult life stages and paid little attention to early life stages or ontogenetic niche shifts. As a result, most Antarctic species are not often framed as cross-system users. Limited, seasonal access and logistical challenges have shaped the trajectory of Antarctic science. In many ways, the depth of our understanding of Antarctic ecosystems lags decades behind that of more temperate and easily accessible systems. While ecological zones remain useful frameworks – especially for describing dominant ecological processes and community structure – and some species are indeed primarily associated with a single zone, observations suggest more direct biological connections across these systems than are broadly recognized. In particular, we posit that sea ice habitat – especially ice reefs, though not exclusively – tightly couples these seemingly disparate zones through ontogenetic habitat and niche shifts. Furthermore, it is known that anchor ice is found up to ~30 m depth and accumulated platelet ice up to ~20 m (Hoppmann *et al*., 2020), yet benthic ice features have traditionally been viewed as disturbance (i.e., ice scouring) rather than a feature of the habitat (Brenner, 2001; Dayton, 1969).

Stages of Ice Reef Emergence

While platelet ice formation and sub-ice platelet layer growth have only been studied in localized regions (e.g., Brett *et al*., 2024; Langhorne *et al.*, 2015), existing research largely focuses on the physical processes underlying ice crystal deposition beneath fast ice. These studies describe how tidal forces, wind events, and supercooled Ice Shelf Water circulation drive seasonal variability in platelet ice accumulation. While these studies have deepened our understanding of physical dynamics of platelet ice formation, the subsequent stages of structural and ecological development – including as integration of anchor ice, structural persistence and flux, or the complex habitat architecture that defines what we refer to here as ice reefs – remain less explored. Similarly, other ice features, such as frazil ice and brinicles, are rarely integrated into ecological frameworks, despite their potential contributions to the structural evolution of macro- and micro-scale ice reef features. Frazil crystals, in particular, may also have important physiological implications by increasing the risk of ice crystal nucleation within biological tissues.

The ice reef lifecycle framework presented here builds on these physical observations by proposing a broader ecological trajectory – from initial formation to functional habitat – that emphasizes the progression from abiotic, passive substrate to ecologically meaningful habitat. We draw on the emergence stages of other reef-like systems, as well as ephemeral ecosystems, to help scaffold a conceptual model of habitat development (**Table 1**). We propose a five-stage ecological cycle of ice reef formation, integrating new observations of structural and biological complexity. While the exact phenological sequence likely varies across regions and taxa, this framework

can both elucidate current understanding of, and catalyze future research on, these systems.

1. **Anchoring**: Reef development begins with some degree of ice stability, typically linked to coastal benthos and landfast ice, which provides anchoring substrate for growing ice features.
2. **Growth**: Platelet ice, anchor ice, brinicles, and frazil crystals accumulate and expand, forming a structurally complex matrix shaped by supercooled water dynamics, tides, and circulation patterns.
3. **Seeding**: Microbial and algal seeding begins within and on the ice matrix, initiating primary production within the reef and eventually giving rise to algal blooms.
4. **Recruitment**: Ice-associated invertebrates and fishes enter the reef, likely seeking refuge, foraging opportunities, or favorable microhabitats.
5. **Disintegration**: Ice reef structures begin to melt and fragment as warm summer conditions destabilize the sea ice and reef matrix, leading to reef disintegration and potentially dispersal.

Fishes' Reliance on Ice Reefs

There are currently seven Antarctic fish species and two Arctic fish species that are described as ice-associated in the literature (**Table 3**). These species have typically been characterized as facultatively associated with sea ice, where their ice-associative behaviors are considered unique exceptions rather than widespread behaviors (Andriashev, 1968; Koubbi *et al.,* 2017; Eastman and DeVries, 1985; Hubold, 1985;

Loeb *et al.*, 1993). However, when viewed through the lens of ecological function, these behaviors suggest broader patterns. Here we review and synthesize the ice-associated behaviors of polar fishes organized by their hypothesized ecological drivers. While ice-associated behaviors are most likely driven by multiple selective pressures, in most cases, the ecological function remains poorly documented and untested. To reflect how our understanding of sea ice ecosystems has evolved, we organize each proposed ecological function in approximate chronological order, based on when they first appear in the literature. We start with early, often speculative interpretations of adult behavior, and end with recent, well-documented examples that underscore the importance of sea ice habitat for early life stages (i.e., ice reefs).

Predation avoidance

One of the earliest and most commonly cited ecological drivers of ice-associative behavior in fishes is predation avoidance. Adult *Pagothenia borchgrevinki* are frequently observed perching in, or swimming near, sea ice cracks (Eastman and DeVries, 1985). Although commonly cited – and a reasonable hypothesis – there is no published evidence of direct observations of *P. borchgrevinki* using ice cracks to avoid seal predation (e.g., Brierley, 2002; DeVries & Steffensen, 2005; Fuiman *et al.*, 2002). There are, however, videos of Weddell seals (*Leptonychotes weddellii*) exhaling bubbles into ice cracks to startle *P. borchgrevinki* into swimming, where they can then snatch the *P. borchgrevinki* (Ainley and Siniff; Randy Davis). This suggests antagonistic coevolution could be driving foraging and hiding behaviors between these quintessential Antarctic predator and prey species.

In the Arctic, evidence for sea ice as predation refugia for adult fishes is somewhat stronger. *Boreogadus saida* (polar cod) have been observed resting on the underside of the sea ice, potentially to conserve energy, as well as in and around pressure ridges and under-ice ridges, potentially avoiding predation from seabirds and marine mammals (Craig *et al*., 1982; Enevoldsen *et al*., 2003; Gradinger and Bluhm, 2004; Lønne and Gulliksen, 1989). Polar cod shoals are strongly associated with ice cover – including drifting pack ice – where they expand their nearest-neighbor distance compared to open waters, where they are thought to shoal more closely as defensive social posturing (Crawford and Jorgenson, 1993). Juvenile and adult life stages of *Arctogadus glacialis* (Arctic cod), a closely related species to polar cod, have also been observed resting on sea ice structures for predation avoidance (Gradinger and Bluhm, 2004; McRoy and Goering, 1974).

Foraging opportunities

Foraging is another likely evolutionary driver of ice-association in fishes (Eastman *et al.*, 1982; Eastman and DeVries, 2007; La Mesa *et al*., 2004; McRoy and Goering, 1974). Several species have been observed foraging in the ice-water interface, suggesting higher, more consistent, or safer availability of food resources. Juvenile *Chionodraco rastrospinosus* have been observed using the ice-water interface as hunting ground for krill furcilia and fish larvae (Moline *et al.*, 2008). *C. rastrospinosus* remains one of the few Antarctic fish with well-documented foraging behavior linked to sea ice structure. While less studied, *T. newnesi* – a dietary generalist – has also been collected in sea-ice associated environments in Terra Nova Bay (Vacchii *et al.*, 2012). Though their

specific interactions with sea ice have not been described, their presence in ice-associated habitats suggest its potential association with foraging behavior.

Larval *Gymnodraco acuticeps* have been observed migrating vertically after hatching from benthic nests, swimming up the water column towards the sea ice, where they are thought to begin to feed (Evans *et al*., 2005). This ontogenetic habitat shift is also presumed to offer predation refugia. *G. acutcieps* exemplify the importance of describing as many life stages as possible; this species is benthic as eggs and adults, but appears to have an ice-associated larval phase – thus a traditionally described benthic fish has a clear, potentially vital, relationship with ice reef habitat.

In the Arctic, *B. saida* feed extensively in the ice-water interface, particularly on ice-associated amphipods and copepods (Crawford and Jorgenson, 1993; Gradinger and Bloom, 2004). Both juvenile and adult life stages have been observed foraging in this zone, underscoring the consistent role of sea ice habitat in feeding across ontogeny. *A. glacialis*, a closely related species, is also found near the sea ice and is thought to forage similarly (McRoy and Goering, 1974; Moline *et al*., 2008).

Ice reef nursery habitat

The most compelling evidence for sea ice providing an essential ecological function for fishes comes from its role as nursery habitat. This function has received growing attention, particularly due to direct *in situ* observations of spawning and early development of fishes in ice reefs.

In Antarctica, *Pleuragramma antarcticum* (Antarctic silverfish) offer a striking, well-documented example. In 2002, researchers at Mario Zucchelli Station in Terra

Nova Bay first documented millions of silverfish eggs, embryos, and larvae encased within, and floating among, the platelet ice matrix accumulated under fast ice (i.e., ice reefs) (Vacchi *et al*., 2004). Subsequent surveys in 2005 and 2006 estimated the nursery habitat size at 270 km^2, encompassing the large area of the Gerlache Inlet and Silverfish Bay adjacent to the Campbell Glacier Tongue in Terra Nova Bay. Concentrations of eggs, embryos, and larvae reached up to 1,100 per liter, and the presence of fishes strongly correlated with the presence of platelet ice. The authors infer that the nursery habitat provides shelter, highly oxygenated water, and abundant nutrients, but this has not yet been empirically demonstrated. Importantly, the sampling method – drilling 15 cm holes through the sea ice and sampling the ice-water interface with a PVC tube – disrupts the structure of the ice reef matrix. This sampling method, while logistically feasible and an excellent choice for assessing the size and density of the nursery, disrupts the ice reef matrix and, with it, our ability to observe behavioral interactions with the ice. We are left with important questions regarding the behavioral adaptations of these fishes and how they interact with the ice on finer scales (i.e., within the ice reef matrix). Given the consistent recurrence of early life stages in this nursery habitat, some suggest that homing behavior could be involved in life history strategies of *P. antarcticum* (Koubbi *et al.*, 2011), however this has not been demonstrated, nor has the exact location and time of spawning. The main spawning hypotheses are that adults either spawn at depth, and eggs float to the surface with frazil ice, or that adults migrate to the shallows to spawn under the ice. Especially given the diversity of egg-laying strategies seen amongst Antarctic fishes, one can imagine other possibilities, like adults

laying eggs in the crevices of the ice reef matrix, or even actively burying their eggs into the platelet ice. At this point, we simply do not know.

While *P. antarcticum* remains the best documented example, there is evidence of several other species potentially using the sea ice as nursery habitat in early development. Larval *G. acuticeps* have been observed immediately swimming upwards towards the surface upon hatching from their egg nests, which are laid 15 – 35 m on flat rocks (Evans *et al*., 2005). Similarly, in 2018 and 2019 at Turtle Rock in the Ross Sea, we observed hundreds of larval *A. dewitti* at 8 – 10 m depth, seemingly swimming upward from depth towards the sea ice, suggesting an ontogenetic habitat shift that may include cryopelagic phases (AJF, pers. obs., 2019). Pelagic *A. dewitti* larvae have also been collected in Terra Nova Bay, although at what depth range is not described (Guglielmo *et al.,* 1998; La Mesa *et al*., 2000). It is currently unknown when, where, and how *A. dewitti* eggs are laid and, generally, extremely little is known about this species. Critically, *A. dewitti* is described as a bathydemersal species, and adults have been collected at depths ranging 146 to 540 m (La Mesa, 2001; Vacchi *et al*., 2001). Our collection of larval life stages in shallow depths of less than 10 m indicates an ontogenetic habitat shift in this species (AJF, pers. obs., 2019; our dive records). Finally, we've also observed and collected post-larval *T. bernacchii* and *P. borchgrevinki* in the shallows of McMurdo Sound, around 3 – 8 m depth (AJF, pers. obs., 2019; our dive records). Together, the observations of *G. acuticeps*, *A. dewitti*, and *T. bernacchii*, while limited, underscore the idea that ice reef habitat may support a critical developmental window for a broader range of species than previously acknowledged, potentially including even bathydemersal species. Significant ontogenetic habitat shifts are

relatively widespread among fishes globally, but such an extreme depth transition would be notable. Such a shift would mean migrating into a challenging environment and risking variable salinity, direct exposure with ice, and increased risk of ice crystal nucleation – which is especially problematic given that larval *G. acuticeps* have relatively low levels of antifreeze proteins (Evans, 2005). It is unknown when and where embryos and larvae of most Antarctic fish species develop (although see: Loeb *et al.*, 1993; Vacchi *et al*., 1999) – much less their levels of antifreeze protein levels – so low levels of antifreeze proteins may or may not be a common challenge across species. Regardless, the benefits – likely feeding and predation avoidance – of such a migration would have to be significant.

We also have some documentation, and substantial *in situ* observations, of juvenile life stages inhabiting ice reefs (**Figure 2**). As researchers focused on early life stages who work out of McMurdo Station, we have spent dozens of hours – with divers who have spent hundreds of hours – exclusively collecting juvenile fishes in McMurdo Sound. We collect within ~20 km of McMurdo Station, going as far as Cape Evans Ice Wall (77°38′23.8″S, 166°31′09.7″E) if sea ice conditions allow. The only habitat where we reliably find juvenile fishes is near-shore, shallow ice reefs (AJF, pers. obs., 2019). And where there are no ice reefs, there are no juvenile fishes (AJF, pers. obs., 2019; R Robbins and S Rupp, pers. comm., 2019). Of course, our observations are limited by depth and divers can only explore down to ~40 m, but we rarely – if ever – find juveniles between 6 and 40 m. Previous authors have also briefly noted diverse benthic and benthopelagic notothens associating with sea ice at larval and juvenile life stages (Knox, 1994; Vacchi *et al.*, 2012). Generally, we find mixed-species assemblages, in

which we find juvenile *T. bernacchii* and *T. pennellii* in abundant numbers, and *T. nicolai* in low numbers. We only sporadically find juvenile *P. borchgrevinki* – in late November of 2018, we found hundreds of juvenile *P. borchgrevinki* just off of McMurdo Station at the intake jetty. In the 2019 field season, we found only four juvenile *P. borchgrevinki* at all dive locations (~100 fishing hours). We have found a handful of other species among the ice reef mixed-species assemblages at the frequented dive locations in McMurdo Sound, including a few juvenile *T. hansoni*, one juvenile *T. newnesi* and one juvenile *P. brachysoma*. In Terra Nova Bay, ~320 km North of McMurdo Sound in the Ross Sea, *T. newnesi* juveniles are thought to enter the water column more frequently if sea ice is present (Daniels and Lipps, 1982; DeVries and Eastman, 1981). Along with *P. borchgrevinki*, and *P. brachysoma*, *T. newnesi* is the only Antarctic species currently described as cryopelagic.

In the Arctic, *B. saida* are known to spawn directly beneath sea ice during the winter months, where eggs and larvae develop in the ice-water interface (Bouchard and Fortier, 2011; Geoffroy *et al.*, 2011). These spawning events often occur in association with pressure ridges or brine channels, which are thought to offer physical protection and stable conditions for embryonic development (i.e., nursery habitat). The reliance of *B. saida* on sea ice for foraging and reproduction throughout ontogeny suggests the importance of sea ice in supporting fish population stability.

Taken together, these observations and quantitative data (while limited), underscore the broader ecological potential of ice reef nursery habitat for a diversity of fishes, including species described as living a life on the benthos. Thus, ice-associated and ice-obligate species may be much more widespread than previously thought,

suggesting a potentially convergent, generalizable life history strategy associated with ice reefs.

Physiological Adaptations to Life in the Ice

The unique physiological traits that allow Antarctic species to survive extreme conditions have fascinated physiologists for decades, and there has been relatively abundant research into these adaptations. *P. borchgrevinki* is the most well-studied species with respect to physiological freezing resistance and cryopelagic adaptations, likely due to relative accessibility; they are regularly observed perched in sea ice cracks in McMurdo Sound, particularly at Cape Evans Ice Wall, where they can be fished with relative ease. Historically, *P. borchgrevinki* has been viewed as a unique cryopelagic specialist (e.g., Andriashev, 1965, 1970; Eastman and DeVries, 1985). Yet a growing number of fishes show similar physiological adaptations and direct physical contact with sea ice, indicating that many species may share a broader ice-associated strategy. Here we review physiological adaptations that enable these fishes to make direct contact with ice, or that indicate a behavioral life history strategy associated with sea ice. We do not include general cold-tolerance adaptations (although see reviews: Cheng and Detrich, 2007; Peck, 2018; Pörtner, 2006; Verde *et al*., 2011). These adaptations highlight that sea ice may be an important driver of polar fish evolution.

An iconic adaptation amongst polar fishes is their ability to synthesize antifreeze glycoproteins (AFGPs), which circulate throughout blood and other extracellular fluids and bind to ice crystals to inhibit their growth after nucleation. This mechanism creates a thermal hysteresis (TH) – a temperature gap between the melting and freezing points – that allows poikilotherms to remain unfrozen in -1.9°C (DeVries, 1971; DeVries and

Wöhlschlag, 1969). This adaptation is particularly well known because AFGPs were first discovered in Antarctic fishes, a revolutionary finding in cryobiology that opened a field of research on ice-binding proteins. This mechanism is seen across fish lineages, as well as other taxa, including crustaceans (*Euphausia superba*), fungi, and bacteria (Deming, 2010; Hoshino *et al*., 2003; Raymond & Kim, 2012; Robinson *et al.*, 2020). Because high-latitude Antarctic seawater remains below the freezing point of biological tissues year round, it is generally assumed that all osmoconforming fauna require some sort of antifreeze mechanism to survive. It is also assumed that fishes with higher AFGP levels may have heightened capacity to interact more closely with sea ice, where the risk of ice nucleation is considerably greater due to supercooled water, high densities of ice microcrystals, and frequent direct physical contact with ice. *P. borchgrevinki* are often cited as having higher levels of AFGPs than other notothens, and this has been interpreted as evidence that *P. borchgrevinki* are particularly adapted for life in the ice (DeVries, 1971; Eastman and DeVries, 1985). Yet, other benthic species that are not considered ice-associated, including *T. bernacchii* and *T. pennellii*, have comparable AFGP levels (DeVries and Cheng, 2005; Wöhrmann, 1996). While TH levels provide a useful metric for freezing avoidance, there is no one definitive threshold at which fish have “enough” AFGPs to maintain contact with ice while preventing freezing. Furthermore, AFGP levels have been shown in various polar fishes to fluctuate through ontogeny (Wöhrmann, 1996) as well as seasonally, where AFGP production is highest in the austral winter when risk of freezing is also highest (Desjardins *et al*., 2006; Fletcher *et al.*, 1984). This plasticity appears to vary across species, and AFGP synthesis is often constitutively high in fishes with persistent exposure to sea ice,

including *P. borchgrevinki*, *T. bernacchii*, and *T. pennellii*. However, many other ice-associated fishes, including eggs and larvae of *P. antarcticum* and *G. acuticeps*, have constitutively low levels of TH – far too low to prevent freezing (Cziko *et al*., 2006). Some of the most compelling examples of ice-association come from species – across life stages – with remarkably low AFGPs – thus high levels of TH are not a reliable criterion for categorizing fish species as ice-associated or ice-obligate. This highlights the need for a more nuanced understanding that considers both physiological measures and observed ecological interactions.

How do early life stages survive life in the ice without sufficient AFGPs? A leading hypothesis is that early life stages rely on morphological barriers to ice nucleation to avoid freezing. Larval *P. antarcticum* and *G. acuticeps*, for example, hatch with underdeveloped gill structures and fully intact integument – traits thought to prevent ice crystals from entering the body in the first place by reducing epithelial exposure to seawater (Cziko *et al*., 2006; Evans and DeVries, 2017). Given the considerable energetic cost of AFGP synthesis, early life stages may rely more heavily on these morphometric defenses because of constrained metabolic resources during growth and development. However, the crossing of the integument by ice crystals is not the only pathway for ice nucleation; ingestion is an open door to ice crystals entering the body. Given that *A. dewitti*, *G. acuticeps*, and *P. antarcticum* begin exogenous feeding early in development (pers. obs.; Vallet *et al*., 2011) – as early as two days post-hatch (Cziko *et al*., 2006) – it remains unclear how these fishes avoid freezing despite insufficient AFGPs, although maternal provisioning of AFGPs is one possible explanation (Cziko *et al*., 2006; Evans and DeVries, 2017).

In addition to AFGP-related adaptations, many Antarctic fishes beautifully camouflage into the sea ice, suggesting an evolutionary relationship with ice habitats. *P. borchgrevinki* and *P. antarcticum* are well known for their silvery-white and pinkish-silver body colorations, respectively, and their silver coloration has typically been interpreted as evidence for a cryopelagic ecotype (Eastman and DeVries, 1985). *P. borchgrevinki* also have dermal xanthophores (specialized photosensitive chromatophores that enable rapid color change), reflective iridophore layers (specialized chromatophores that reflect light), and “inconspicuous” eyes that make them almost disappear into the ice when viewed from below (Eastman and DeVries, 1985; Obika and Meyer-Rochow, 1990). This camouflage involves a suite of crypsis mechanisms, including background matching, reflectivity, and motionless behavior, that together enable passive visual concealment in the structurally and optically complex ice reef.

‘Benthic’ *T. bernacchii* were the first fish species found to contain dermal xanthophores (Obika and Meyer-Rochow, 1990). This mechanism conceals the fishes within the ice reef, presumably to aid in predation avoidance, and may help them quickly move between ice and rock substrate. This would be an elegant adaptation that could enable these fishes to take advantage of both the ice reef and the rocky benthos, greatly expanding their available habitat range. Adult *T. bernacchii*, *T. pennellii*, and *T. nicolai* are rather dark in color, similar to the volcanic rock substrate covering the shelf benthos. But early life stages of these fishes are very light in color – often nearly translucent – such that they disappear into the ice reef matrix (**Figure 2**; AJF, pers. obs., 2019). When diving, we really can only visually detect juveniles using bright underwater flashlights that illuminate their reflective iridophores (AJF, pers. obs., 2019). This

becomes particularly necessary under dim light conditions caused by thick sea ice, heavy snowpack, and/or overcast weather.

The question of if predators can detect the juveniles sitting in the ice can, conveniently, be explored by examining their own spectral sensitivity, as older/larger fishes are likely their primary predators. Antarctic fishes inhabit a uniquely dim, blue-shifted light environment under persistent sea-ice, as well as complete darkness in the austral winter (MacDonald and Montgomery, 1991). Notothenioid visual systems appear to be specialized for these conditions – they cannot see red or orange wavelengths, which are filtered within the first few meters of seawater, even without ice cover (Carleton *et al*., 2020; Pointer *et al*., 2005). Notothenioids have poor visual acuity to see fine-scale details, but enhanced sensitivity to detecting subtle contrast changes, meaning they could easily perceive a silhouette of prey swimming, but not fine detail such as outlines or textures (Castiglione *et al*., 2023; Morita *et al*., 1997; Montgomery *et al.*, 1989; Pankhurst and Montgomery, 1989). Notably, they have some ability to see momentary UV glints – which their own dermal iridophores reflect (Castiglione *et al*., 2023; Carleton *et al*., 2020; Pointer *et al*., 2005). Thus it is likely that adult notothenioids have the ability to see their smaller juveniles counterparts if they are swimming, but not when they are sitting within the ice reef matrix. This strongly supports the hypothesis that ice reefs provide nursery habitats for young notothenioids.

Together, the biochemical, structural, and morphological traits found across notothenioid species and life stages indicate a broader pattern of ice-associated physiological adaptation. However, some physiological metrics, such as AFGP concentration, do not necessarily align with observed behaviors, particularly across

ontogeny. And in many cases, we still cannot explain how some species and life stages survive their icy habitat, indicating we still have much to learn about polar fish physiology, particularly with respect to habitat specialization.

Ice-Association in Fishes: Not Just A Unique Specialization?

Historical definitions of ice-association in Antarctic fishes have largely been shaped by early studies focused on adult physiological traits — often conducted by a relatively small cohort of researchers working within limited geographic and seasonal windows. These frameworks have profoundly influenced how ecotypes are described and which species are considered “adapted” to sea ice. Yet mounting behavioral evidence across life stages reveals that many species may interact with sea ice in more common, ecologically meaningful, and dynamic ways than have been widely appreciated. Defining species ecotypes primarily through a physiological lens risks overlooking ecologically significant behaviors that may be critical in survival and reproduction – particularly when their physiological profiles do not align with preconceived expectations of what ‘should’ be necessary for ice-association.

Ice-association and ice-obligation are distinct life history strategies. While ice-associated species use sea ice for part of their development, ice-obligate species depend on it for survival, with limited or no ecological alternatives. Recognizing this distinction has important implications for ecological forecasting under climate change, where the loss of sea ice may affect species differentially based on the degree of their dependency on ice (Post *et al*., 2013). We suggest that the terms ‘cryopelagic’ and ‘cryobenthic’, though useful in describing habitat zones, are limited in their ecological

specificity. These characterizations do not capture the relative importance or necessity of sea ice in a species' life history strategy – nor do they allow room for ontogenetic shifts in habitat use. In contrast, ice-associated and ice-obligate more clearly communicate the ecological role that sea ice plays across stages of development – and across taxa. These terms reframe ice association not as a rare specialization, but as a potentially widespread ecological strategy shaped by consistent interactions with a dynamic habitat.

Refugia Habitats

The interaction of early life stages with sea ice has been viewed by many physiologists as "just a temporary trophic relationship" (e.g., Eastman & DeVries, 1985) and even evidence of behavioral failure, in which larvae are "thwarted in their attempts to escape the icy surface layer because of its three-dimensional complexity" (Evans & DeVries, 2017). This assumption – that the presence of young fishes in ice habitats is incidental rather than adaptive – overlooks the possibility that their consistent occurrence reflects ecological selection. Rather than viewing sea ice as an obstacle to survival, we suggest that the repeated occurrence of early life stages in this zone – paired with physiological and morphological adaptations – indicates that ice habitat may be selected and ecologically important, not an accidental trap. Under a reef framework – in which we reframe sea ice as a living, structured, reef-like habitat rather than simply an abiotic ceiling overhead – its ecological opportunities become crystal clear. Ice reefs are a functional landscape, offering substrate, shelter, nutrients, and potentially favorable

temperature microclimates. Within this framework, we highlight the important role of providing refugia and nursery habitat for many polar fishes.

More authors are beginning to call attention to the importance of sea ice for certain species, and we argue that this emphasis is both overdue and essential to a more nuanced view of sea ice as a living biome. The possibility that ice reef habitat provides refugia is meaningful not only for individual early life stages, but also for population- and community-level dynamics. Spatial refugia are known to promote predator-prey stability (Sih, 1987), provide feeding grounds for young stages (Verweij *et al.*, 2006), enhance biomass (Mumby *et al*., 2004), and increase biodiversity (Keppel *et al.*, 2011). Some have even argued that refugia are the most critical driver of prey species abundance (Andrewartha and Birch, 1984; Berryman and Hawkins, 2006; Errington, 1946) – a dynamic that becomes clear when we shift from our standard predator-centric perspective to a prey-centric perspective (Berryman and Hawkins, 2006).

Refugia habitats are exemplified in marine ecosystems, where we see flourishing, diverse communities centered around coral reefs, eelgrass meadows, kelp forests, mangrove roots, oyster reefs, rock reefs, and sargassum rafts (**Table 2**). These refugia habitats serve as safe havens for young and/or vulnerable species. The ecological importance of refugia habitat becomes evident when they are lost. Without refugia, populations can reach an “extinction threshold” – where the metapopulation doesn’t disappear immediately after the habitat loss, but instead collapses a few generations later (Nee and May, 1982). This creates “extinction debts” (Tilman et al., 1994), in which the current metapopulation does not reflect the true longevity of their

constituent populations. The intergenerational and population-level consequences of habitat loss emphasizes the importance of focusing on nursery habitat for young life stages.If a diversity of Antarctic fishes depend on ice reef habitat as refugia, then ice-limited future oceans could increase their predation risk and reduce biodiversity and abundance, as seen in other ecosystems (Greggor *et al*., 2016).

Ice Reef Phenology

Sea ice is a highly dynamic environment in constant flux across spatiotemporal scales. It is tightly coupled with oceanographic, atmospheric, and geophysical processes, including currents, tides, wind, and photoperiod, which shape sea ice through both short-term variability (i.e., weather) and long-term trends (i.e., climate). Because of this constant flux, sea ice characteristics (e.g., extent, depth, ice reef complexity, snowpack, and stability) demonstrate significant stochasticity and interannual variability. This dynamism is an important ecological and evolutionary driver that shapes the life history strategies of organisms across taxa that depend on sea ice habitat for substrate, nutrients, foraging, reproduction, development, and/or survival (Ainley and Tynan, 2006; Tynan *et al.*, 2010). Understanding the phenological relationships of organisms with sea ice is an important area of research both in the Arctic and Antarctic, and is particularly urgent as climate change amplifies and disrupts these systems (Cimino *et al.*, 2022; Post *et al.*, 2013; Swadling *et al*., 2023). Climate change is delaying sea ice formation, accelerating break out, and drastically reducing sea ice extent and age (particularly in the Arctic); all of these changes lead to significantly reduced sea ice stability (Schofield *et al*., 2024). The consequences of altered sea ice phenology cascade through the

ecosystem, including reduced primary productivity, altered nutrient cycling, habitat loss, phenological mismatch, trophic rewiring, community instability and loss of biodiversity (Corso *et al*., 2022, Massom and Stammerjohn, 2010; Post, 2017; Ramírez *et al*., 2017).

Ice reefs, as structurally and ecologically integrated features of the broader sea ice system, are inherently shaped by the same oceanographic, atmospheric, and geophysical processes and also demonstrate spatiotemporal stochasticity and interannual variability. They are ephemeral habitats in constant flux, growing and shape-shifting through the seasons. Critically, the spatial extent of ice reefs remains entirely unknown; unlike surface sea ice, current satellite technology cannot resolve subsurface ice features, and known locations of ice reefs are essentially limited to diver observations and footage from ROVs. What we do know is that ice reef habitat is spatially patchy and appears to be concentrated in regions near ice shelves and glacier tongues, which are thought to promote platelet ice formation (Hoppmann *et al*., 2020). Bathymetric features, landfast ice stability, brine rejection, and katabatic winds are also important drivers in platelet ice, anchor ice, and brinicle formation – the key components of ice reef structure (Cartwright *et al*., 2013; Hoppmann *et al.*, 2020; Petrich and Eicken, 2010; Weeks, 2010). Given that both ice shelves and glacier tongues are broadly distributed around the periphery of the Antarctic continent (Luchitta *et al.*, 1993; Rignot *et al.*, 2011), patchy ice reefs could reasonably provide extensive circumpolar developmental habitat for fishes and other taxa. Assessing the extent and characteristics of ice reef habitat surrounding Antarctica remains a challenge and offers

a crucial line of future research to evaluate the vulnerability of nearshore Antarctic ice reef communities to climate change and identify regions of potential resilience.

The patchiness of ice reefs is echoed in the patchiness of young fish assemblages. In some species, we have observed interannual patterns in occurrence in ice reefs, while other species' occurrence is stochastic across years and sites (Vacchi *et al*., 2012; our dive records). We hypothesize that ice reefs may cue, constrain, or facilitate reproductive success depending on seasonal phenological dynamics. Ice reefs exhibit both macro- and micro-scale structural complexity and we see substantial heterogeneity within the reef, on the order of several meters to just a meter or less, down to the fine-scale architecture of the platelet ice matrix itself. This heterogeneity and complexity may create distinct microhabitats within the reef matrix. Like tide pools in the rocky intertidal and coral reef microhabitats, these pockets vary in temperature, salinity, light availability, nutrient concentration, and food availability and may create ephemeral spaces of refuge, food resources, and/or metabolic buffering for developing fishes. We have some evidence of heterogeneous species composition in young fish assemblages in microhabitat pockets within ice reef habitat in McMurdo Sound (AJF, pers. obs., 2019; our dive records). The availability of micro- and macro-habitats is necessarily tied to the seasonal timing of sea ice dynamics, including the duration, persistence, and timing of formation, disintegration, and breakout of ice reefs, as well as macro- and micro- structural complexity. Such factors would shape the spatial and temporal windows in which these habitats function as effective refugia and nurseries. While necessarily conceptual, this framework is grounded in over 40 hours of *in situ* observations, supported by dive haul records, photography and videography, and

ongoing discussions with researchers and divers who know these habitats intimately from thousands of diving hours. Moreover, this framework is grounded by other described ice-dependent reproductive phenologies in fishes, including walleye (*Sander vitreus*) and Antarctic silverfish (*P. antarctica*) (Barta *et al.*, 2024; Corso *et al*., 2022), as well as across taxa in both the Arctic and Antarctic (for a review, see Tynan *et al*., 2010).

Thus far, seasonal sea ice access has extremely limited our observations of these systems; everything we know is based on a few months in the austral summertime, at only a handful of locations, and by very few people. Our hope is that Antarctic field research will [[continue to be funded :')]] so that we can explore these questions more deeply.

Ice Reef Habitat Loss Compounds with Multiple Co-occurring Stressors

The ice reef framework fundamentally reframes how we understand nearshore structured sea ice zones – not as treacherous obstacles to escape, but as potential refugia and nursery habitat. This shift invites us, as observers, to reconsider our current understanding of the life history strategies of a diverse group of Antarctic fishes and to draw attention to a habitat at acute risk of disintegration as climate change amplifies. Sea ice is disappearing at a pace that exceeds most ensemble climate model projections – models that already predict widespread sea ice loss by 2100 (Stroeve *et al*., 2012; Post, 2017). While climate change is rapidly melting sea ice in both polar regions, the Arctic is warming faster via Arctic amplification (Screen and Simmonds, 2010; Serreze and Barry, 2011). Multi-year sea ice (MYI) is now extremely rare in the

Arctic as it is replaced by annual ice; MYI extent is at around 5% of 1980's MYI extent (Meier *et al*., 2024). Projections for Antarctic sea ice are much less clear and sea ice extent has grown in many regions where it was modelled to shrink (Liu, 2025). Critically, even with unexpected sea ice growth in some regions, projected and observed sea ice loss is not spatially uniform – some regions of the Antarctic are expected to experience localized increases in sea ice extent, while others show sharp declines (Jena *et al*., 2023; Liu, 2025; Nie *et al*., 2023). Irrespective of direction, climate change is altering both sea ice dynamics and phenology, which increases the risk of reduced recruitment, population and community instability, and trophic mismatch. That risk intensifies if ice reefs are, in fact, essential nursery habitat. Reduced larval abundance of *P. antarcticum* has already been linked to both warmer sea surface temperatures and reduced sea ice (Corso *et al.*, 2022). At present, the distribution and extent of ice reefs remain completely unknown – much less how climate change may alter their growth, stability, and ecological functions. Ice reef structural features depend on supercooled conditions; it is therefore reasonable to assume that warming trends will reduce both the occurrence and structural persistence of ice reefs – especially in regions experiencing ice shelf retreat and reduced fast ice duration.

Of course, habitat loss is only one of the many environmental changes threatening species as climate change continues to amplify. Ice-associated and ice-obligate species face a suite of co-occurring climate change stressors, including ocean warming, acidification, and altered salinity regimes. There has been relatively abundant research on the physiological effects of ocean warming on Antarctic fishes (see: **Chapter 3**; Beers and Jayasundara, 2015; Bilyk and DeVries, 2011; Todgham and

Mandic, 2020) and a modest amount on ocean acidification (Davis *et al*., 2016, 2018; Enzor *et al*., 2013, 2017; Flynn *et al*., 2015; Hancock *et al*., 2020; Naslund *et al*., 2021; Strobel *et al*., 2012, 2013) and freshening (Navarro *et al*., 2019; O'Grady and DeVries, 1982; Spencer *et al*., 2020; Vargas-Chacoff, 2016). However most of these studies have examined only one environmental condition. Research has consistently shown that co-occurring stressors usually interact non-additively, and can be synergistic or antagonistic (Breitburg *et al*., 2015; Crain *et al*., 2008; Darling and Côté, 2008; Hofmann and Todgham, 2010; Todgham and Stillman, 2013). As such, it is difficult to accurately predict species responses to co-occurring stressors without studying them concurrently – going forwards, it will be critical to examine how ice reef habitat loss may interact with predicted thermal, acidification, and salinity regimes.

For ice-associated and ice-obligate fishes – that spend significant time in the ice-water interface – freshening and osmoregulatory stress is of particular concern (Kültz, 2015). Antarctic fishes are generally considered stenohaline, with limited physiological capacity to tolerate salinity fluctuations (DeVries and Steffensen, 2005). In contrast, Arctic fishes tend to show greater salinity tolerance, likely due to long-standing adaptation to riverine freshwater input (Cheng and Dietrich, 2007; Kijewska *et al*., 2016; Małachowicz *et al*., 2023; Spencer *et al*., 2020). In other ecosystems, climate-driven habitat loss and physiological stressors have been shown to interact synergistically, accelerating biodiversity declines (Mantyka-Pringle *et al*., 2011; Nagelkerken, 2016). Furthermore, qualitative network models predict that with reduced sea ice duration, pelagic algae, copepods, krill, and fish will all decrease in abundance alongside increases in salp abundance – a shift that would fundamentally disrupt trophic dynamics

in the Southern Ocean (Swadling *et al*., 2023). The potential loss of ice reef nursery habitat may have cascading effects on species persistence and population- and community- stability. Understanding the degree to which early life stages of Antarctic fishes rely on ice reef habitat is essential to assessing which species may be able to persist under rapidly changing environmental regimes.

Conclusions

Fishes are critical players in polar ecosystems. Through their roles in energy transfer, trophic stabilization, and bentho-pelagic coupling, fishes influence food web dynamics across trophic levels, habitat zones, and seasons. Habitat loss for juvenile notothenioids is expected to have cascading effects on the marine ecosystem (Kortsh *et al.*, 2015). If ice reef habitats serve as essential nurseries for developing fishes, then their loss could disrupt not only individual species trajectories but also broader community and ecosystem stability.

Physiological and behavioral data suggest that polar fishes do not end up in sea ice habitat incidentally. Across life stages – from larvae to juveniles to adults – a diversity of polar fishes show repeated behavioral patterns of moving toward the ice, despite the physiological challenges that this habitat is thought to impose, including risk of freezing and osmoregulatory stress. These repeated behavioral patterns of ice-association, especially at early life stages, suggest that the ecological and/or physiological benefits of ice reefs – such as predation refugia, abundant nutrients and food, and/or more preferable microclimates – outweigh the risks of migrating to and/or

surviving close contact with ice. The trade-offs involved imply that sea ice habitat may play a far more central role in polar fish life histories than previously recognized.

Ice reefs represent a structurally complex, ephemeral, and climate-vulnerable habitat that may be essential for the persistence of polar fish populations. As sea ice continues to decline, understanding the extent to which polar fishes rely on ice reef habitats – particularly during early development – is critical for predicting which species may persist under future environmental regimes and which niche shifts may occur. Ice reefs may also inform the design and placement of marine protected areas (MPAs), especially if they promote biodiversity.

While *P. borchgrevinki* and *P. antarcticum* have historically been viewed as unique examples of benthic species that evolved to exploit cryopelagic habitat, we suggest that this transition may not be exceptional. Assuming the ancestral notothenioid stock was indeed benthic, the repeated apparent behavioral and physiological associations with ice reefs across species and life stages imply that this shift toward sea ice may reflect a broader ecological strategy. Rather than a handful of rare evolutionary departures, ice-association and ice-obligation may have provided recurring ecological opportunity and shaped the life histories of many Antarctic fishes. Moreover, the repeated use of sea ice habitat across diverse taxa, especially during early development, suggests that sea ice may exert consistent selective pressures. We posit that sea ice may not merely be tolerated, but may be preferred habitat – even required – by certain species during key developmental windows. In this light, sea ice may be recognized not just as harsh challenges, but as potential evolutionary drivers not of

hardship alone, but also of shelter and nourishment that may scaffold the beginnings of life for young Antarctic fishes.

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Table 1. Glossary of terms.

Term	Explanation	References
Albedo	Refraction of light reflected by a surface or object; measured on a scale from 0-1	Petrich and Eicken, 2010
Anchor ice	Ice attached to the benthos	Hoppmann *et al.,* 2020;
Antifreeze glycoproteins (AFGPs)	Proteins in the blood that bind to ice crystals to inhibit their growth after nucleation	DeVries, 1971; DeVries and Wöhlschlag, 1969
Arctic amplification	Extreme temperature variability and warming trends observed in the Arctic region, upwards of 3x as severe as the global average	Serreze and Barry, 2011; Screen and Simmonds, 2010
Benthic	Organisms inhabiting the sediment (e.g., sand, rocks, mud) at the bottom of a body of water	
Brine rejection (brine exclusion)	The process of salt being expelled from seawater as it freezes, because salt molecules do not fit in the crystal lattice of frozen water	Lake and Lewis, 1970; Notz and Worster, 2008
Brinicle	A hollow, columnar sea ice feature, similar to an icicle or stalactite in appearance, that grows downward from the sea ice, sometimes reaching the benthos, due to brine rejection	Cartwright et al., 2013
Cryobenthic	Species found beneath ice shelves	Bornemann *et al*., 2018a; Bornemann *et al*., 2018b
Cryonotothenioid	A subgroup of notothenioid fishes endemic to the Southern Ocean that are specialized to exist in a narrow range of near-freezing temperatures.	Corso *et al.,* 2024
Cryopelagic	Species found directly under sea ice; used to describe ice-associated fishes	Andriashev, 1970; Eastman and DeVries, 1985; Mel'nikov, 1989

Cryophilic	Cold-loving organisms; generally used to describe higher-order organisms (animals and plants) that thrive in cold habitats	
First-year sea ice (FYI)	Sea ice that has formed during the most recent winter and has not yet survived a summer melt season; typically 0.3 m – 2 m thick; saltier and weaker than MYI	
Frazil ice	Fine crystals of ice suspended in the water column	Tsang and Hanley, 1985
Furcilia	Krill in their final larval phase, after the calyptopsis stage before entering juvenile stage	
Glacier	A large, perennial mass of frozen ice and snow, often containing rock and sediment debris, that originates on land and flows downslope due to gravity	
Glacier tongue (ice tongue)	A long, narrow, floating extension of a glacier that can project from the calving front out onto the water surface	
Ice-associated species	Species that rely on sea ice for feeding, resting, and/or reproduction; expected to experience decreased fitness from disappearing sea ice	Moore and Huntington, 2008; Macias-Fauria and Post, 2018
Ice-obligate species	Species that interact with sea-ice for feeding, resting, and/or reproduction, but are not thought to be reliant; expected to face trade-offs and increased competition due to disappearing sea ice	Moore and Huntington, 2008; Macias-Fauria and Post, 2018
Ice sheet	A massive body of glacial ice covering more than 50,000 km^2, up to 5 km thick and millions of years old	
Ice shelf	A think, floating extension of a continental glacier or ice sheet that forms where land-based ice flows out to the ocean	
Ice-water interface	The dynamic boundary layer where the underside of ice meets the liquid water below	

Incorporated platelet ice	Solid sea ice resulting from the consolidation and freezing of the sub-ice platelet layer	Hoppmann *et al.,* 2020;
Katabatic winds (catabatic)	Downsloping winds driven by the pressure gradient force and gravitational force created when an elevated cool, high-density air mass flows into a lower density air mass	Parish and Bromwich, 1987
Landfast ice (fast ice)	Sea ice attached to the coastline, ice shelf, or grounded icebergs; does not move with currents or winds	Weeks, 2010
Meiofauna	Small organisms 50 – 1000 μm in size; often include copepods, flatworms, nematodes, rotifers, turbellarians; usually associated with the benthos	
Multi-year sea ice (MYI)	Ice that has survived at least one summer melt season; typically 2 – 4 m thick	
Nearest neighbor distance (NND)	The shortest straight-line distance between a fish and its closest neighboring fish within a group or school	
Nutricline	The water layer within which nutrient levels decline rapidly with depth; zone containing the chlorophyll maximum	Cermeño *et al*., 2008
Pagophilic	Ice-loving organisms that prefer or require ice for feeding, resting, or reproduction; includes ice-associated and ice-obligate species	
Pelagic	Organisms that occupy the open sea and/or water column	
Platelet ice	A specific form of ice characterized by thin, plate-like ice crystals on the centimeter to decimeter scale. Platelet ice grows *in situ* in supercooled waters, often underneath ice shelves. This general term may encompass platelet ice crystals, the sub-ice platelet layer, and/or incorporated platelet ice	Hoppmann *et al.,* 2020;
Platelet ice crystal	An individual piece of platelet ice	Hoppmann *et al.,* 2020;

Pycnocline	Transitional layer between a warmer, less-dense surface water mass and the colder, denser, and deeper water mass below it	
Sea ice	Frozen sea water that floats on the ocean surface; FYI or MYI; usually up to ~10 m thick	Weeks, 2010
Sea ice extent	The area of ocean where at least 15% of the surface is frozen	
Stenothermal	Tolerance to only a narrow range of temperatures	
Sub-ice platelet layer	Layer of individual platelet crystals (can be several meters thick) accumulated underneath solid sea ice cover; may be mobile or static	Gow *et al*., 1998; Hoppmann *et al.,* 2020;
Sympagic	Organisms or processes that occur within ice structure; often used to describe meiofauna in sea ice	
Thermal hysteresis (TH)	The temperature differential between melting and freezing points	

Table 2. Comparative structurally complex reef-like ecosystems

Habitat	Reef Description	Ecosystem Functions	References
Coral reefs	Three-dimensional (3D) $CaCO_3$ skeletons built by scleractinian corals in tropical, shallow waters	· Create a hard-substrate skeleton, 3D habitat complexity, and refugia · Nurseries for young fishes · Support high biodiversity · High primary productivity · Nutrient cycling and carbon deposition · Enhance water quality · Shoreline protection	Komyakova *et al*., 2013; Nagelkerken *et al*., 2000; Wilson *et al*., 2010; Woodhead *et al*., 2019
Eelgrass meadows (seagrass beds)	Dense underwater meadows of flowering seagrasses (e.g., *Zostera*, *Thalassia*) anchored by extensive rhizome networks in shallow coastal sediments	· Create 3D habitat complexity and refugia · Nurseries for young fishes and invertebrates · Support high biodiversity · Nutrient filtration, improved water quality · Carbon sequestration · High primary productivity · Sediment stabilization and erosion control · Shoreline protection	Costa *et al*., 1994; Nagelkerken *et al*., 2000; Nagelkerken *et al*., 2002; Unsworth *et al*., 2019; Waycott *et al*., 2009; Whitfield, 2017; zu Ermgassen *et al*., 2021
Kelp forests	Dense stands of large brown macroalgae (e.g., *Macrocystis*, *Laminaria*) anchored to rocky substrates in cold, nutrient-rich subtidal zones	· Create 3D habitat complexity and refugia · Nurseries for young fishes and invertebrates · Support high biodiversity · Carbon sequestration · High primary productivity · Nutrient cycling · Shoreline protection	DeMartini *et al*., 1989; Holbrook *et al*., 1990; James and Whitfield, 2022
Mangrove roots	Intertidal networks of aerial and underwater roots of mangrove trees along tropical and subtropical shorelines	· Create 3D habitat complexity and refugia · Nurseries for fishes and crustaceans · Pollutant filtration · Nutrient cycling · Carbon sequestration · Storm surge protection	Mumby *et al*., 2004; Nagelkerken *et al*., 2000; Nagelkerken *et al*., 2002; Polidoro *et al*., 2010; Rogers *et al*., 2016; Whitfield, 2017

Oyster reefs	Biogenic reefs formed by aggregations of oyster shells and live oysters in intertidal and shallow subtidal estuarine zones	· Create hard substrate skeleton, 3D habitat complexity, and refugia · Habitat for epifaunal communities · Support high biodiversity · Enhance water quality · Sediment stabilization and erosion reduction · Carbon sequestration	Coen *et al.,* 1999; Gilby *et al*., 2018; zu Ermgassen *et al.,* 2021
Rock reefs	Ridges of rocky substrate in subtidal oceans	· 3D habitat complexity and refugia · Substrate for sessile invertebrates and macroalgae · Nurseries for young fish and invertebrates · Support high biodiversity	Cheminée *et al*., 2017; Dance *et al*., 2021; Grol *et al*., 2014; Jones, 1988
Salt marsh beds	Tidal wetlands dominated by halophytic grasses (e.g., *Spartina*) with dense root mats in estuarine shorelines	· Create 3D habitat complexity and refugia · Nurseries for young fishes and invertebrates · Support high biodiversity · Sediment trapping and shoreline stabilization · Water filtration · Nutrient cycling · Carbon sequestration · Wave energy attenuation	Costa *et al*., 1994; Rogers *et al.,* 2016; Whitfield, 2017; zu Ermgassen *et al*., 2021
Sargassum rafts	Free-floating mats of pelagic macroalgae (e.g., *Sargassum*) drifting in open-ocean gyres and coastal currents	· Create 3D habitat complexity and refugia · Nurseries for young fishes and invertebrates · Support high biodiversity · Facilitate long-distance dispersal · High primary productivity · Promote carbon cycling	Alleyne *et al*., 2023; Martin *et al.*, 2021; Safran, 1990; Stachowiak, 2020

Table 3. Potential ice-associated or ice-obligate polar fish species

Species	Pole	Location	Life stage	Ice-associated behavior	Ice type	References
Acanthodraco dewitti	Antarctic	McMurdo Sound	Larvae	Swimming upwards to sea ice?	Ice cover?	AJF, pers. obs., 2019
Arctogadus glacialis	Arctic	Bering Sea	Juvenile, adult	Resting, feeding, predation avoidance?	Ice cover	McRoy and Goering, 1974
Boreogadus saida	Arctic	Canada Basin; Bering Sea	Adult	Resting, feeding, predation avoidance	Ice cover	Craig *et al*., 1981; Enevoldsen *et al*., 2003; Gradinger and Bluhm, 2004; Lønne and Gulliksen, 1989; McRoy and Goering, 1974
Chionodraco rastrospinosus	Antarctic	Antarctic Peninsula	Larvae	Feeding	Ice cover?	Loeb *et al*., 1997; Moline *et al*., 2008; Quetin *et al*., 1996
Gymnodraco acuticeps	Antarctic	McMurdo Sound	Larvae	Feeding?	Ice cover?	Evans *et al*., 2005
Notothenia coriiceps	Antarctic	Bransfield Straight	Eggs, embryos	Nursey habitat, spawning?	Ice cover	Kellerman, 1996; Moline *et al*., 2008; Sapota, 1993, 1999
Pagothenia borchgrevinki	Antarctic	McMurdo Sound	Juvenile, adult	Resting, predation avoidance and feeding?	Ice reefs; sea ice cracks of fast ice	AJF, pers. obs., 2019; Kellermann, 1996
Pagothenia brachysoma	Antarctic	Balleny Islands	Adult	Resting, feeding	Drifting ice floes	Andriashev, 1987; Andriashev, 1970; Ślósarczyk, 1983
Pleuragramma antarcticum	Antarctic	Terra Nova Bay	Eggs, embryos, larvae	Nursery habitat (shelter, feeding?), spawning?	Platelet ice layer (ice reefs?)	Daniels and Lipps, 1982; Vacchii *et al*., 2004

Trematomus bernacchii	Antarctic	McMurdo Sound	Juvenile	Resting, predation avoidance?	Ice reefs	AJF, pers. obs., 2019
Trematomus newnesi	Antarctic	West Antarctic Peninsula	Juvenile	Feeding	Landfast ice	Daniels, 1982; Daniels and Lipps, 1982; Kellermann, 1996; Vacchii *et al*., 2012
Trematomus nicolai	Antarctic	McMurdo Sound	Juvenile	Resting, predation avoidance and feeding?	Ice reefs	AJF, pers. obs., 2019
Trematomus pennellii	Antarctic	McMurdo Sound	Juvenile	Resting, predation avoidance and feeding?	Ice reefs	AJF, pers. obs., 2019

Figures

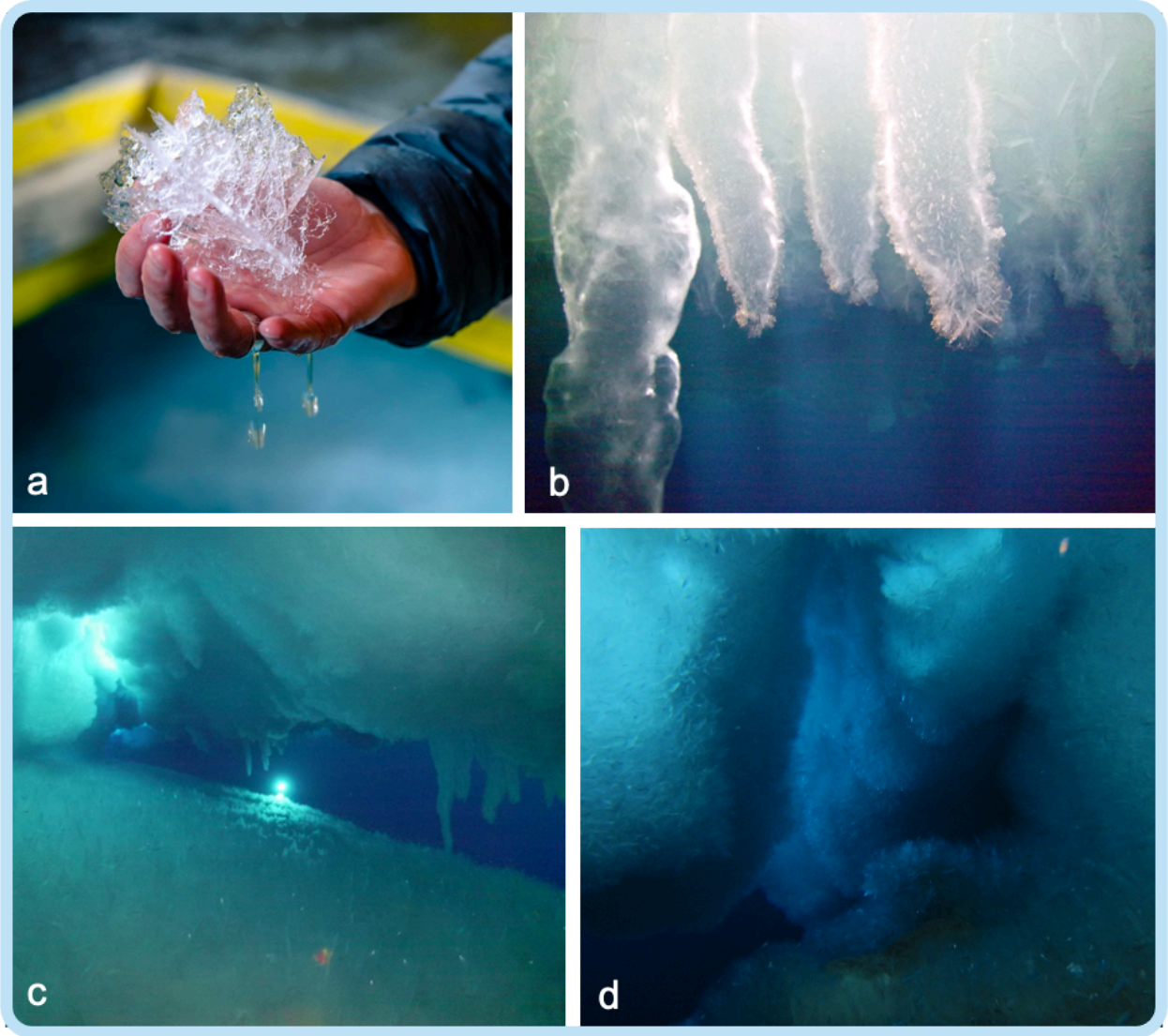

Figure 1. Micro-, intermediate-, and macro-scale ice reef features. Panel (**a**) depicts a large platelet ice crystal, panel (**b**) depicts brinicles, panel (**c**) depicts the near-shore ice reef comprised of brinicles, anchor ice, and complex reef structure growing on the underside of the sea ice. Panel (**d**) depicts the common intermediate-scale complexity of ice-reef structure. Photos by Kira Morris (**a**, 11/19 at Turtle Rock), Steve Rupp (**b**, 11/13 at Arrival Heights; **c**, 10/13 at Arrival Heights) and Rob Robbins (**d**).

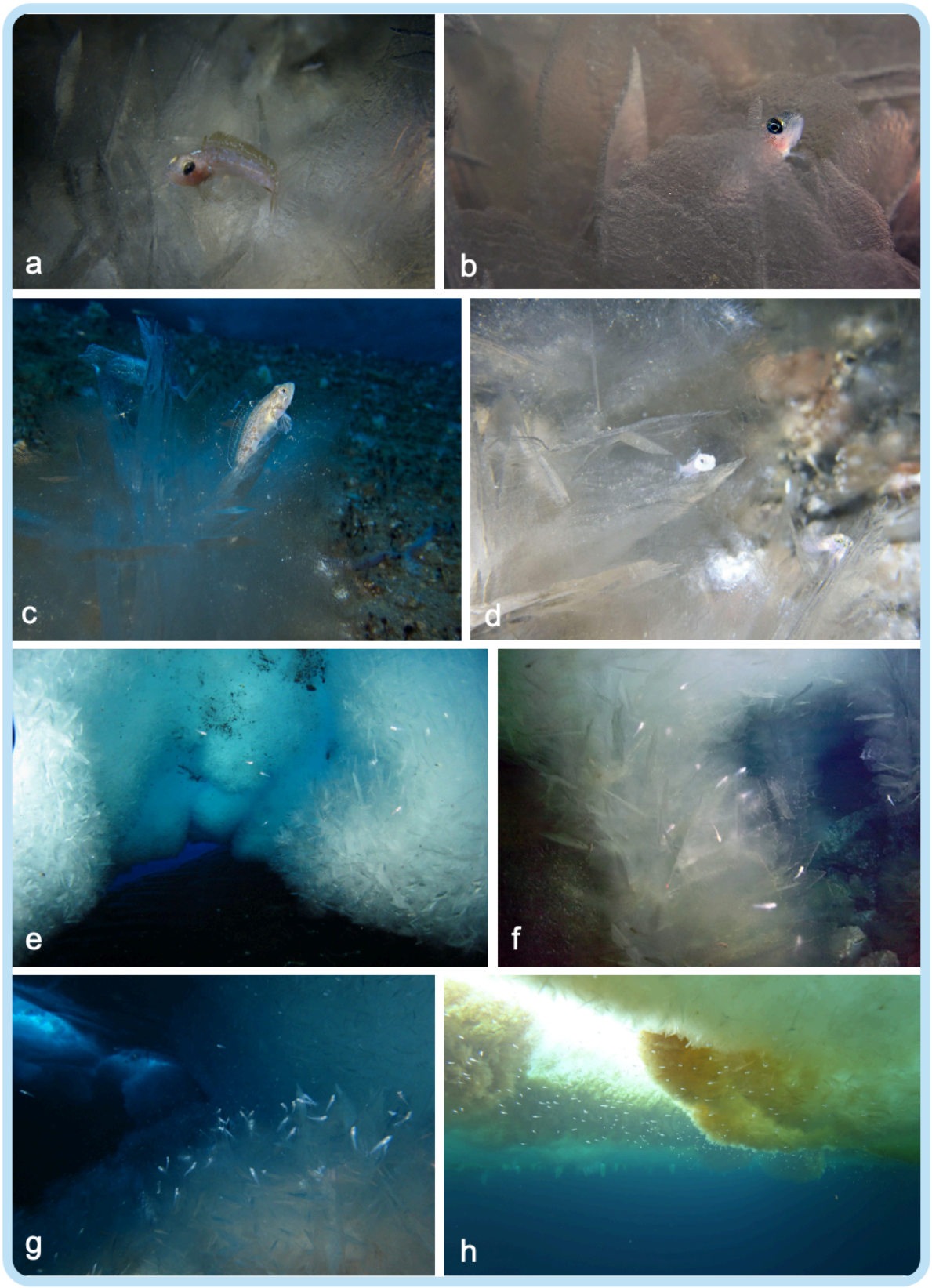

Figure 2.

Figure 2. Juvenile Antarctic Notothenioid fishes in ice reef habitat in McMurdo Sound. Panels (**a** – **d**) depict micro-scale ice reef features and common resting behaviors across juvenile Notothenioid species observed by SCUBA divers. Panels (**e** – **h**) show the complex intermediate and macro-scale ice reef structure; the silver streaks are all juvenile Notothenioid fishes resting in the sea ice. Photos by Rob Robbins (**a**, 11/13 at the jetty; **c**, 11/13 at Dayton's Wall; **d**, 11/13 at the jetty; **e**, 10/19 at Turtle Rock; **g**, 12/13 at the jetty), Paul Cziko (**b**), and Steve Rupp (**f**, 11/13 at Cape Evans Ice Wall; **h**, 11/19 at the jetty).

CHAPTER 5

General Discussion

Overarching Aims, Revisited

The overarching aim of my PhD research was to better describe how juvenile Antarctic fishes may respond to climate change stressors characteristic of predicted future oceans. I used a comparative approach rooted in niche theory to examine species-specific vulnerability to climate change. I focused on understanding niche differentiation among species, as well as potential behavioral strategies that may support species coexistence and metabolic strategies that may inform species capacity to cope with stressors such as climate change. Together, my goal was to help improve predictions of species-specific vulnerability to climate change stressors. Due to important physiological, behavioral, and ecological dynamics, studying early life stages is essential to characterizing sensitivities that affect the entire population.

I first examined interspecific niche differentiation of four abundant juvenile Antarctic fishes under ambient conditions: *Trematomus bernacchii*, *Trematomus pennellii*, *Trematomus nicolai*, and *Pagothenia borchgrevinki*. By examining behavioral and physiological niche differentiation under ambient conditions, we can gain a better understanding of ecological dynamics in these fish assemblages that may be impacted by climate change. In doing so, we can make predictions about what species may be "winners" and which may be "losers" as climate change amplifies. This can help us identify species of particular concern to prioritize in future studies. I focused on basal metabolic traits and the exploration-avoidance axis of behavior, two key dimensions of

species fitness and in defining an organism's niche (Brown *et al*., 2004; Ern, 2019; Gvoždík, 2018; Hamilton *et al.*, 2021; Pörtner *et al*., 2010; Rodriguez-Dominguez *et al.*, 2022; Sih *et al.*, 2011, 2012; Smith and Blumstein, 2008). I found similar energetic physiology across the four species (assessed with resting metabolic rate and body condition), but differential exploratory strategies when exposed to novelty (assessed with the Novel Object Test and Novel Tank Test); *T. bernacchii* and *T. pennellii* showed risk-prone behavior, *T. nicolai* were avoidant, and *P. borchgrevinki* explored cautiously. I also found substantial intraspecific variability in behavioral traits, suggesting the presence of divergent behavioral phenotypes within species. This work emphasizes that divergent behavioral strategies are key drivers of niche differentiation in Antarctic fish assemblages and could shape species-specific performance under environmental threats.

Using my first comparative study as a foundation, Chapter Three focused on behavioral strategies of two species from Chapter Two, *T. bernacchii* and *T. pennellii*, under ocean acidification (OA) and ocean warming (OW) climate change stressors. *T. bernacchii* and *T. pennellii* are two closely related species abundant in the near-shore McMurdo Sound. It's generally thought that these species occupy very similar niches; they coexist in the shallow benthos as adults, school together in mixed-species assemblages in ice reefs as juveniles, and have generalist diets (**Chapter 4**; Eastman and Lannoo, 1995; La Mesa *et al.*, 2004; Montgomery *et al*., 1993). Their similarities in trophic niche and habitat use can lead to an implicit assumption that they may respond to stressors in similar ways, but this assumption is overly simplistic and may mask important differences in their ecological strategies and stress responses. In Chapter

Two, *T. bernacchii* and *T. pennellii* both demonstrated relatively risky behaviors. Given that OAxOW stressors are known to alter behavior – particularly behaviors on the exploratory-avoidant axis – I was interested to better understand how climate change may alter risky behavior across species, and if *T. bernacchii* and *T. pennellii* would show similar or differential behavioral responses once under stressors. I focused on the exploratory-avoidant axis of behavior using the Novel Object Test (NOT) to assess if OAxOW acclimation impacts exploratory activity or induce anxiety and/or neophilia (i.e., interest in novel objects). Over five weeks of acclimation, warming was the primary driver of behavioral change in both species. Elevated pCO_2 did not significantly alter *T. pennellii* behavior and only showed subtle effects in *T. bernacchii* that dissipated early within the acclimation period. Although I did not find statistically significant interactions between OA and OW, observed trends do suggest a potential interaction that warrants further research. Both species reduced exploratory activity and increased neophilic behavior under the warming condition. These responses amplified over time, and *T. pennellii* demonstrated a stronger response (i.e., effect sizes) in both behavioral metrics. Consistent with previous physiological and behavioral studies, while limited, these results support the hypothesis that *T. pennellii* have a particularly risky strategy when faced with novelty that is amplified when acclimated to warming. It is currently unclear if a riskier strategy is adaptive or maladaptive in this ecosystem. Chapter Two and Chapter Three together emphasize that behavior is a critical dimension of the stress response and may drive interspecific vulnerabilities.

While diving and collecting our fishes, I became captivated by the role that sea ice habitat plays for these species. It seemed evident to me – as a ‘predator’ trying to

catch fishes – that sea ice provides vital nursery habitat across notothenioids. Yet only a handful of polar fishes have been described as ice-associated, and generally an ice-associative strategy has been regarded as an interesting, unique exception, rather than the expectation, for polar fishes. Based on dozens of hours of my own *in situ* observations, conversations with other Antarctic scientific divers with decades of experience, video recordings, previous literature, and nursery habitat use across species globally, I think that an ice-associative or ice-obligate strategy is far more prevalent and ecologically significant than has been characterized. In Chapter Four, I propose the idea of viewing sea ice habitat as 'ice reefs'. The reef framework is valuable, because I speculate that ice reef structure plays a similar role as structured habitats in more temperate systems (e.g., coral reefs, kelp forests, oyster reefs, sargassum rafts). In these habitats, abiotic and biotic components together create three-dimensional habitat complexity that act as skeletons – they provide attachment sites, shelter, and stability and promote the rich biodiversity that nursery habitats foster. Similarly, sea ice microstructure can be thought of as the skeleton of the sea ice reef – it provides a complex structure where micro and macrofauna thrive. Understanding sea ice habitat in a reef framework emphasizes that the ice itself is integral to the ecosystem.

An important theme of this dissertation is that understanding species-specific responses to climate stressors requires bridging physiological ecology, behavioral ecology, and habitat dynamics within the larger ecological context. Ontogeny is a critical intersection point; developmental shifts influence physiological stress responses, behavioral strategies, and ultimately their broader ecological niche. This work further

highlights that subtle differences in behavior and physiology may be related in divergent responses to climate change stressors. For example, in Chapter 2, *T. pennellii* demonstrated the riskiest behavioral strategy under ambient conditions, and the stress-induced amplification of risky behavior was notably stronger in *T. pennellii* than *T. bernacchii*. This finding exemplifies the value of niche theory in making predictions of species resilience – or vulnerability – to environmental change.

Climate Change Threatens Ice Reefs

The ice reef framework introduced in Chapter Four offers an essential ecological context for interpreting our findings on how species respond to climate change stressors. Ice reefs, similar to more temperate structured habitats like coral reefs, kelp forests, and oyster reefs, provide three-dimensional complexity and crucial nursery habitats. In turn, ice reefs may facilitate stable predator-prey interactions, trophic stability, and biodiversity. As climate change further intensifies sea ice loss, Antarctic species – especially those reliant on ice-associated habitats – may face significant stress and displacement. Like other reef-like habitats, sea ice habitat relies on abiotic conditions (e.g., temperature, salinity, ocean chemistry) that integrate with biotic dynamics, thereby creating potentially essential niches that may underpin ecological stability. Not only would the stress of habitat loss likely amplify stressors in abiotic conditions (e.g., OA, OW, changes in salinity) within individuals (i.e., contributing to physiological distress), it would likely also have reverberating ecological consequences (Kortsch *et al*., 2015; Walther, 2010). Here I focus on the potential larger ecological consequences and look to other, more temperate reef-like habitats as examples of

ecosystems that have undergone phase shifts related to climate change induced die-off of structured habitat. I briefly discuss the examples of coral reef, kelp forest, and oyster reef phase shifts from habitat degradation. By examining other reef-like habitat phase shifts, we emphasize the interconnected relationship between structured habitats and community stability; their collapse, driven by warming, ocean acidification, and habitat degradation, has frequently resulted in dramatic ecological regime shifts and altered ecosystem states. Ultimately, we see a pattern in which the structural complexity itself is critical (Alvarez-Filip *et al*., 2011; Alvarez-Filip *et al.*, 2013; Castaño *et al*., 2021; Komyakova *et al*., 2013).

In coral reef ecosystems, there are many well-documented examples of phase shifts into persistent algal cover resulting from reef degradation and mortality due to bleaching and heat stress, disease, and/or over-fishing of herbivores (Clements and Hay, 2018; Darling and Côté, 2013; Gardner *et al*., 2003; Hughes *et al.*, 2003; Mumby, 2006; Mumby and Steneck, 2008; Pendleton *et al*., 2016). When reef-building corals die, the reef loses it's three-dimensional structure is 'flattened', reducing habitat complexity and refugia for reef organisms (Alvarez-Filip *et al*., 2009; Alvarez-Filip *et al*., 2011; Alvarez-Filip *et al.*, 2013; Castaño *et al*., 2021; Komyakova *et al*., 2013). Algae proliferate on dying reefs and further hinder coral reef recovery, leading to persistent algal dominance (Clements and Hay, 2018; Darling and Côté, 2013; Hughes et al., 2003; Mumby and Steneck, 2008; Pendleton *et al*., 2016). Algal-dominated states reduce biodiversity, ecological function, ecosystem maturity, and ecosystem services of the reef ecosystem (Ainsworth and Mumby; 2015; Komyakova *et al*., 2013; Wilson *et al.*, 2006; Woodhead *et al*., 2019). The diversity and richness of fish assemblages,

particularly but not exclusively those that rely on reef-building corals, are negatively impacted by degradation of the three-dimensional structure of coral reefs (Ainsworth and Mumby; 2015; Castaño *et al*., 2021; Wilson *et al.*, 2006).

Oyster reefs once formed extensive, complex three-dimensional habitats that supported rich biodiversity (historical oyster reefs supported hundreds of macrofaunal species) and key ecosystem services across temperate regions, akin to coral reefs (Beck *et al*., 2011; Grabowski and Peterson, 2007; Thurstan *et al*., 2024; zu Ermgassen *et al*., 2012, 2024). But overexploitation, destructive fishing (i.e., dredging), disease, and pollution have led to the collapse of 85% of oyster reef ecosystems globally, where the three-dimensional structure of the oyster reef has been flattened, resulting in a phase shift to an essentially barren seafloor (Gillies *et al.*, 2018; Thurstan *et al*., 2024). In many areas, wild oyster populations remain below 10% – and are often closer to 1% – of their historic abundance, leaving these habitats functionally extinct (Schulte *et al*., 2009). Coastal ecosystems have undergone fundamental restructuring as a result, with turbid water, reduced fisheries, and eroded natural coastal defenses replacing the once-biodiverse reef state with decimated biodiversity and ecosystem functioning (Gillies *et al.*, 2018).

Kelp forests around the world have also experienced rapid and devastating regime shifts driven by compounding stressors and trophic cascades. Overfishing and extirpation of apex predators (e.g., sea otters, lobsters, and large reef fishes) release herbivorous urchins from predation, triggering population booms and intensive grazing that deforests kelp (Rogers-Bennett and Catton, 2019; Steneck *et al*., 2002; Wilman, 2021). These phase shifts are reinforced by self-reinforcing feedback loops, where

intensive urchin grazing suppresses kelp recruitment and, in turn, habitat complexity. Poor habitat complexity minimizes predator recovery, allowing urchin populations to continue unchecked grazing, thus maintaining a stable urchin barren state (Filbee-Dexter and Scheibling, 2014). Ocean warming and marine heatwaves further weaken kelp, and pollution (i.e., eutrophication, sedimentation, poor water quality) and severe storm disturbances can decimate kelp canopies, exacerbating kelp loss (Connell *et al.*, 2008; Filbee-Dexter & Scheibling, 2014). The collapse of kelp habitat results in profound ecological consequences, where diverse and productive kelp forests that once supported rich communities and provided nursery grounds for fishes are now replaced with urchin barrens, characterized by low biodiversity, diminished primary productivity and carbon storage, and fundamentally altered trophic dynamics (Pérez-Matus *et al.*, 2025; Steneck *et al.*, 2002). Kelp forest to urchin barren phase shifts have been documented across temperate oceans, from California and Alaska to New Zealand, Tasmania, and Japan, illustrating a global degradation of structured coastal ecosystems (Carnell and Keough, 2020; Filbee-Dexter and Scheibling, 2014; Ling, 2008). Eventually, the urchins may decimate the ecosystem to the extent that they themselves begin to starve (Filbee-Dexter and Scheibling, 2014).

Coral reefs, oyster reef, and kelp forest degradation and die-off exemplify a common pattern in structured reef-like systems – the loss of a foundation species and its three-dimensional structured habitat triggers feedback mechanisms that entrench the ecosystem in a degraded, ‘wicked’ state (Alvarez-Filip *et al.*, 2009; DeFries and Nagendra, 2017; Geerling *et al.*, 2007; Ling *et al.*, 2009; Nyström *et al.*, 2012; Perkol-Finkel and Airoldi, 2010; Scheffer *et al*., 2001). In each case, the structured

habitat was providing critical ecological functions, including creating refugia and feeding grounds, and buffering physical conditions, which maintained ecosystem stability, biodiversity, richness, and resilience (Coen *et al*., 2007; Scheffer *et al*., 2001). Similar dramatic state changes are seen in many other structurally complex ecosystems, including mangrove roots, seagrass beds, and salt marshes (Moffet *et al*., 2015; Whitaker *et al*., 2022; Wimmler *et al*., 2021). In all of these examples, once the structure erodes, key processes that kept the ecosystem balanced are disrupted, allowing opportunistic organisms to dominate and further impede the original habitat's recovery, holding the ecosystem in an alternative stable state with low complexity (Alvarez-Filip *et al*., 2009; Ling *et al*., 2009; Mumby *et al.*, 2007; Nyström *et al.*, 2012). These 'wicked' states are characterized by reduced biodiversity, diminished ecosystem services, and strong hysteresis. Often, life essentially dies off completely (Ling *et al*., 2009; Scheffer *et al*., 2001). Wicked ecosystem states are difficult to reverse without significant intervention, because the very absence of complex structural habitat makes the system more vulnerable and less resilient to further disturbances (Folke *et al.*, 2010; Nyström *et al.*, 2012). Wicked ecosystem states of what were previously thriving reef-like ecosystems results in significant losses to ecosystem services, including nursery habitat for fishes, water filtration, shoreline stabilization, and nutrient cycling (Gillies *et al.*, 2018; Nagelkerken *et al.*, 2000; Schulte *et al*., 2009).

Looking to the diverse reef-like ecosystems collapsing into wicked stables states across the world raises alarm bells for ice-reefs and the species that rely on them. Given rapid sea ice loss and co-occurring alterations to environmental conditions (particularly ocean warming, ocean acidification, and freshening), ice reefs may be in a

precarious position. Furthermore, if juvenile life stages of many notothenioid fishes, including those studied here, may depend on sea ice, shifts in this habitat could have profound consequences for population, community, and ecosystem stability.

The important role of structural complexity urgently calls for situating stress physiology studies within realistic ecological contexts, particularly structured habitats, to improve predictions of organismal performance under co-occurring climate change stressors (Pendleton *et al*., 2016; Smith *et al.*, 2024). Given the role of sea ice in modulating biogeochemical cycles, such as seasonal CO_2 fluxes creating habitat for primary producers, changes in sea ice regimes will alter carbon cycling dynamics, potentially exacerbating other stressors and impacting organisms' physiological and behavioral responses (Arrigo, 2010; Delille *et al*., 2014; Fransson *et al*., 2013; Miller *et al.*, 2011; Rysgaard *et al*., 2011). Mesocosm and field experiments may help integrate more complex habitat changes in experimental biology studies.

This work also highlights the critical role of ontogeny in shaping how Antarctic fishes respond to climate change. Early life stages are often the most sensitive to environmental stressors and are crucial in determining population dynamics and resilience. Given that egg, larval, and juvenile notothenioid fishes likely rely on sea ice reefs as nursery habitats (**Chapter 4**), their developmental success and survival may be intimately connected to ice reef habitat stability. Warming and reduced sea levels have already been shown to decrease reproductive success of *Pleuragramma antarcticum*, a critical fish in Antarctic marine food web (Corso *et al.*, 2022). Other reef-like, structurally complex systems exemplify the importance of resources required at specific ontogenetic stages (Beck *et al*., 2001; Holbrook *et al.*, 1990; Nagelkerken, 2000; Pérez-Matus,

2023). Given that physiological tolerances, behavioral strategies, and habitat preferences often change markedly through life stages, future stress physiology studies that explicitly integrate ontogenetic shifts may help uncover species-specific vulnerabilities to climate change-driven habitat loss and environmental change.

Rapidly accelerating rates of sea ice loss, ocean freshening, and warming amplify risks of disrupted predator-prey dynamics, phenological mismatches, and trophic collapse, all of which heighten the likelihood of irreversible ecological shifts (Kubelka *et al*., 2022; Iler *et al*., 2021; Inouye, 2022; IPCC, 2021; Rintoul *et al*., 2018; Thackeray *et al*., 2016). Antarctic ecosystems may already be approaching critical thresholds, where relatively small further perturbations – such as increased competition from temperate species migrating poleward, altered nutrient cycling, or increased disease prevalence – could initiate regime shifts and biodiversity loss. This research advocates for an integrative, comparative ecological approach to studying potential ecosystem responses to climate change. By combining physiological and behavioral frameworks with explicit ecological contexts (i.e., ice reef habitat), this dissertation provides a strong foundation for future studies. It is urgent that we study ice reef habitats before they disappear altogether.

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