Integrative and Comparative Biology

Integrative and Comparative Biology, volume 60, number 6, pp. 1414–1424
doi:10.1093/icb/icaa083

Society for Integrative and Comparative Biology

SYMPOSIUM

Pinniped Ontogeny as a Window into the Comparative Physiology and Genomics of Hypoxia Tolerance

Emma L. Weitzner, Cornelia E. Fanter and Allyson G. Hindle [1]

School of Life Sciences, University of Nevada Las Vegas, Las Vegas, NV 89154, USA

From the symposium "New Frontiers in Antarctic Marine Biology" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2020 at Austin, Texas.

[1]E-mail: allyson.hindle@unlv.edu

Synopsis Diving physiology has received considerable scientific attention as it is a central element of the extreme phenotype of marine mammals. Many scientific discoveries have illuminated physiological mechanisms supporting diving, such as massive, internally bound oxygen stores and dramatic cardiovascular regulation. However, the cellular and molecular mechanisms that support the diving phenotype remain mostly unexplored as logistic and legal restrictions limit the extent of scientific manipulation possible. With next-generation sequencing (NGS) tools becoming more widespread and cost-effective, there are new opportunities to explore the diving phenotype. Genomic investigations come with their own challenges, particularly those including cross-species comparisons. Studying the regulatory pathways that underlie diving mammal ontogeny could provide a window into the comparative physiology of hypoxia tolerance. Specifically, in pinnipeds, which shift from terrestrial pups to elite diving adults, there is potential to characterize the transcriptional, epigenetic, and posttranslational differences between contrasting phenotypes while leveraging a common genome. Here we review the current literature detailing the maturation of the diving phenotype in pinnipeds, which has primarily been explored via biomarkers of metabolic capability including antioxidants, muscle fiber typing, and key aerobic and anaerobic metabolic enzymes. We also discuss how NGS tools have been leveraged to study phenotypic shifts within species through ontogeny, and how this approach may be applied to investigate the biochemical and physiological mechanisms that develop as pups become elite diving adults. We conclude with a specific example of the Antarctic Weddell seal by overlapping protein biomarkers with gene regulatory microRNA datasets.

Introduction

Marine mammals exert profound yet fine-scale cardiovascular control during submergence, and at the same time protect their cells from hypoxia and/or ischemic damage. The elite diving mammalian phenotype centers around oxygen, its storage, metabolic use, and maintenance of a protective cellular milieu. Despite understanding the physiological building blocks that support diving, we lack mechanistic knowledge of the underlying molecular and genetic elements of this extreme phenotype. Here we present a review of the development of dive physiology in maturing pinnipeds (seals, sea lions, and walruses), focusing on protein-level changes that define the maturation of body oxygen stores, metabolism, and cell protection. While adult pinnipeds are elite divers, pups are born on land. This physiological transition across development is much larger in pinnipeds compared to cetaceans or sirenians, whose calves are born in water. Despite the remarkable phenotypic maturation that pinnipeds undergo, both pups and adults share a genome. This transition therefore presents an experimental framework in which we can investigate comparative physiology using a single-species genomic toolkit.

Multi-species physiological comparisons have been successful in uncovering phenotypic nuances, yet with limited availability of high-quality annotations, similar multi-species comparative genomic approaches remain difficult. Furthermore, the number of species needed to generate evolutionarily relevant data is high, as it is difficult to parse

Advance Access publication June 19, 2020

Table 1 Comparisons of developmental phenotypes using genomic approaches

Species	Approach	Method platform	Phenotype	Citation
Austrofundulus limnaeus	sncRNA profiling	Illumina HiSeq 2000	Anoxia tolerance	Riggs and Podrabsky (2017)
Austrofundulus limnaeus	post-translational modifications of histone H3	ELISA and Western Blots	Diapause	Toni and Padilla (2016)
Austrofundulus limnaeus	mRNA profiling	Illumina HiSeq 2000	Diapause	Romney et al. (2018)
Crysemys picta	mRNA profiling	Illumina HiSeq 4000	Anoxia tolerance	Fanter et al. (2020)
Crotalus simus simus	mRNA profiling, miRNA profiling, Proteomics	454-Pyrosequencing, Ion Torrent, reverse-phase HPLC	Venom composition	Durban et al. (2013)
Homo sapiens	mRNA profiling	Illumina HiSeq 3000	Cardiac development	Pervolaraki et al. (2018)
Leptonychotes weddelli	miRNA profiling	Illumina HiSeq 2500	Diving	Penso Dolfin et al. (2020)
Gallus gallus, Mus musculus, Rattus norvegicus	lncRNA profiling	Illumina HiSeq 4000	Role of lncRNAs in development	Darbellay and Necsulea (2020)

phenotype-specific signals from noise due to interspecies variation. An initial effort to identify the genetic signatures of convergent evolution across sirenians, pinnipeds, and cetaceans used 4 species and identified 43 genes with convergent changes in these 3 marine mammal lineages (Foote et al. 2015). Subsequent work expanding the analysis to 54 mammals revealed false positives in the initial analysis (Thomas et al. 2017). Multi-species genomic studies may yield limited insights if convergent phenotypes are not similarly regulated at the molecular level. Indeed, it appears that similar pathways, but few exact genetic changes, define convergence in the three marine mammal lineages (Chikina et al. 2016). A survey of small noncoding RNA (sncRNA) expression from four divergent anoxia tolerant species also found little conserved expression (Riggs et al. 2018). Similarly, few commonly expressed genes were detected in transcriptomes among high-altitude species (Hao et al. 2019). Moreover, multi-species approaches impose challenges for functional follow-up studies as it is difficult to develop and validate molecular tools in every species. Therefore, to investigate extreme phenotypes with a modern molecular and genomic toolkit, single-species developmental comparisons may be invaluable. We begin by reviewing examples of studies where developmental comparisons provided key insights into species-specific physiology (Table 1).

Case studies: Single-species studies elucidating phenotype via ontogeny

The annual Killifish (*Austrofundulus limnaeus*)

The annual killifish is a model for stress tolerance, including anoxic exposure and desiccation (Wourms 1972; Podrabsky et al. 2001). This species annually experiences drought. Adults do not survive, whereas the *A. limnaeus* embryo persists for weeks by entering a metabolically depressed state of diapause (Podrabsky et al. 1997). Embryos can enter diapause at three distinct developmental timepoints, and with the recent assembly of a sequenced genome (Wagner et al. 2018), developmental comparisons within this species have been used to pinpoint the cellular cues responsible for diapause and associated stress responses. For example, a global survey of temperature-induced transcription in dormant and developing embryos identified differential expression of genes associated with Vitamin D3 synthesis (Romney et al. 2018). This discovery led to a series of investigations that revealed the importance of Vitamin D signaling in vertebrate development and in cuing dormancy. Examination of the epigenetic marks associated with embryonic arrest at different developmental timepoints indicated that post-translational modification patterns in histone H3 reflected altered development (Toni and Padilla 2016). A role for mitochondria-derived small ncRNAs in regulating reactive oxygen species during recovery from hypoxia was uncovered by comparing sncRNA expression across development (Riggs and Podrabsky 2017).

The painted turtle (*Chrysemys picta*)

Adult painted turtles have a 4-fold higher anoxia tolerance than hatchlings (Reese et al. 2004; Dinkelacker et al. 2005; Odegard et al. 2018). Adults annually experience months of anoxia overwintering in ice-locked ponds, while hatchlings overwinter in terrestrial nests, surviving sub-zero temperatures (Storey et al. 1988; Ultsch 1989). Leveraging the annotated *C. picta bellii* genome (Shaffer et al. 2013), this model has elucidated

tissue-specific cellular mechanisms that define these contrasting phenotypes. Cardiac transcriptomes identified development-specific gene expression patterns during anoxia; for example, microRNA (mRNA) expression of a suite of ribosomal proteins decreased in adults but increased in hatchlings, suggesting that transcriptionally-controlled suppression of translation may support the anoxia-tolerant phenotype (Fanter et al. 2020).

Central American Rattlesnake (*Crotalus simus* simus)

A single-species genomic approach has been applied to gain toxicological insights into changes in venom composition with maturation. As rattlesnakes age, they produce more snake venom metalloproteinases (SVMP), resulting in a venom with less neurotoxicity (Gutiérrez et al. 1991; Calvete et al. 2010). This species has been used as a model to isolate the development-specific changes responsible for altered venom composition. A survey of both gene and protein expression in adults and juveniles found that adults showed high expression of mRNAs that targeted crotoxin-B while juvenile-specific mRNAs targetingPIII-SVMP mRNA were in high abundance, mirroring the known venom compositional shift from crotoxin-rich (juvenile) to SVMP-rich (adult) (Durban et al. 2013). Comparing these development-specific phenotypes revealed the importance of post-transcriptional regulation in venom composition.

Traditional models

There has also been a push to use omics-based tools to characterize tissue-specific, developmental changes in traditional models. Developmental changes in mRNA expression have been characterized in human cardiac tissue (Pervolaraki et al. 2018) and the role of long noncoding RNAs(lncRNAs) during development has been explored in the brain, liver, kidney, and testes of mice, rats, and chickens (Darbellay and Necsulea 2020). With more studies characterizing the fundamental patterns of development in traditional models, it will become easier to filter out conserved developmental signals to focus solely on the novel components of species-specific phenotypes.

Pinniped ontogeny: Biochemistry and physiology to omics

Pinniped pups are physiologically less competent divers than adults; they have reduced mass-specific oxygen stores, higher mass-specific metabolic rates, and are less able to initiate and regulate the cardiovascular dive response (e.g., Burns and Castellini 1996; Burns 1998; Greaves et al. 2004; Noren et al. 2005; Spence-Bailey et al. 2007). Omics studies could provide key insights into genetic regulation of these features, along with other, previously unsuspected aspects of phenotype. While several omics studies (Boaz et al. 2012; Champagne et al. 2013; Khudyakov et al. 2015; Khudyakov et al. 2017) have explored key stages of development in the northern elephant seal, *Mirounga angustirostris* (e.g., the post-weaning fast), similar experiments targeting pinniped diving physiology are less common. To date, the ontogeny of diving physiology is primarily understood through protein biomarkers (metabolic enzymes, respiratory pigments, and structural proteins). Here, we present a review of the current state of knowledge of the biomarkers of pinniped ontogeny, and suggest that integration of single-species genetic or genomic approaches with these data will point to molecular regulation of the diving phenotype.

Oxygen stores

Pups have consistently lower total and mass-specific oxygen stores compared with adults (Table 2). Blood constitutes the largest internal oxygen store; otariids store ~54% of oxygen in blood and phocids store ~65% (Ponganis et al. 2011). Most pinniped neonates are not born with mature hematology (Table 2) and in fact experience declines in mass-specific blood oxygen stores over the nursing period due to rapid gains in body mass (Noren et al. 2005). This physiologic anemia of infancy, well-documented in terrestrial animals, results from a decreased proportion of hemoglobin per unit blood volume caused by a rapid expansion of plasma volume (Burns et al. 2004; Burns et al. 2005, 2007; Noren et al. 2005; Clark et al. 2006, 2007; Weise and Costa 2007). Mature blood oxygen values take a minimum of 3 months to develop in phocids (e.g., Thorson and Le Boeuf 1994) and a minimum of 9 months in otariids (e.g., Verrier et al. 2011). Noren and Edwards (2020) provide a comprehensive review of the measured hematological parameters in the pinnipeds. Uniquely among pinnipeds, 2-week-old walrus (*Odobenus rosmarus*) calves show mature hematology. This could be because walrus calves join their mothers in the water merely days after birth (Noren and Edwards 2020).

Diving mammals have 10–20× the concentration of muscle myoglobin than terrestrial mammals (Ponganis et al. 2011; Mirceta et al. 2013). Pinnipeds, especially otariids, begin life with myoglobin stores similar to terrestrial animals (Fowler et al. 2007; Spence-Bailey et al. 2007; LaRosa et al. 2012)

Table 2 Internal oxygen stores (mean ± SEM, mL $O_2 \cdot kg^{-1}$) in blood, muscle, and total body (TBO_2) between four phocids, four otariids, and walrus

		Oxygen (mL $O_2 \cdot kg^{-1}$)				
Species	Age	Blood	Muscle	TBO_2	Percentage of adult	Source
Harbor seal *Phoca vitulina*	Neonate	45.6 ± 5.2	4.0 ± 0.6	51.8 ± 7.1	75	Burns et al. (2005)
	Nursing	31.2 ± 1.3	5.9 ± 0.5	39.4 ± 2.1	57	
	Adult	38.4 ± 2.4	14.7 ± 1.1	68.7 ± 4.1	–	
Hooded seal *Christophora cristata*	Neonate	48.6 ± 3.2	6.9 ± 0.5	57.7 2.7	64	Burns et al. (2007)
	Nursing	41.8 0.5	7.3 ± 0.3	57.1 ± 0.3	64	
	Adult	45.9 ± 2.6	37.2 ± 1.0	89.5 ± 3.0	–	
Harp seal *Pagophilus groenlandicus*	Neonate	33.8 ± 8.7	3.7 ± 0.1	39.4 ± 8.7	55	Burns et al. (2007)
	Nursing	18.8 ± 1.4	6.5 ± 0.6	28.8 ± 1.9	40	
	Adult	37.9 ± 3.5	29.7 ± 1.2	71.6 ± 3.4	–	
Gray seal *Halichoerus grypus*	Neonate	30.8 ± 3.6	4.2 ± 0.1	38.1 ± 3.7	62	Noren et al. (2005)
	Nursing	21.0 ± 0.9	5.1 ± 0.7	30.7 ± 1.1	50	
	Adults	41.5 ± 4.5	15.9 ± 1.3	61.4 ± 4.6	–	
California sea lion *Zalophus californianus*	5-month	9.4 ± 0.8	11.1 ± 0.7	28.7 ± 1.4	56	Weise and Costa (2007)
	Adult female	22.3 ± 1.0	21.0 ± 0.5	51.1 ± 1.1		
Stellar sea lion *E. jubatus*	1-month	10.1	2.3 ± 0.1	20.6 ± 0.1	51	Richmond et al. (2006)
	5-month	62.2 ± 4.1	5.5 ± 0.3	26.2 ± 0.9	65	
	Adult female	18.2 ± 1.0	14	40.4 ± 1.0	–	
Australian fur seal *A. pusillus*	9-month	–	–	–	71	Spence-Bailey et al. (2007)
Northern fur seal *Callorhinus ursinus*	<1 month	–	–	–	66	Shero et al. (2012)
Walrus *O. rosmarus*	Neonate	–	7.5	–	–	Noren and Edwards (2020)
	Adult		21.6 ± 0.7			

Note: % adult is the percent of adult TBO_2. Dashes indicate unavailable or not applicable information. Mean ± SEM data were taken from cited papers where they were presented, which contain sample size information.

(Table 2). By 9 months, myoglobin concentrations are only 21% of adult values in Australian fur seals, *Arctocephalus pusillus* (Spence-Bailey et al. 2007). Yet, blood oxygen parameters approach adult levels by this age, so myoglobin development may constrain total body oxygen stores (Spence-Bailey et al. 2007). Similar patterns occur in other otariids; by 15 months, Australian sea lions (*Neophoca cinerea*) have adult-level blood oxygen stores, yet only 48% of adult myoglobin (Fowler et al. 2007). Hematocrit and hemoglobin of Steller sea lions (*Eumetopias jubatus*) approach adult levels by 9 months, whereas muscle oxygen stores take 17 months to reach adult levels (Richmond et al. 2006).

Iron forms the heme core of oxygen binding pigments, including muscle myoglobin and blood hemoglobin, making iron a necessary element of oxygen store development (Burns and Hammill 2008; Geiseler et al. 2013). Many pinnipeds undergo a post-weaning fast (Burns et al. 2004), thus, endogenous iron stores are required to synthesize heme proteins (Burns and Hammill 2008; Geiseler et al. 2013). Hooded seal (*Cystophora cristata*) pups have higher liver iron contents and concentrations than older animals (Geiseler et al. 2013), suggesting the liver as the primary iron source for heme protein biosynthesis. Early anemia may result from an iron intake insufficient to meet expanding demands, leading to a slow development of circulating and muscle oxygen stores during early nursing (Burns and Hammill 2008).

Whereas iron availability constrains the development of oxygen stores, hypoxia exposure, and exercise training may spur them. Kanatous et al. (2008) found Weddell seal (*Leptonychotes weddellii*) pups have lower swimming muscle myoglobin than adults, but the highest myoglobin concentrations for this species occur in juveniles (1- to 2 year olds). This

may be driven by juveniles' mounting exposure to hypoxia with increased diving activity, which stimulates myoglobin synthesis *ex vivo* (De Miranda et al. 2012). Hypoxia exposure and exercise training have been correlated with increased myoglobin in terrestrial, semi-aquatic, and marine mammals (Stephenson et al. 1989; MacArthur 1990; Saunders and Fedde 1991; Morrison et al. 1966; MacArthur et al. 2001). In addition to the need for large iron reserves for myoglobin biosynthesis, the delay in the exposure of pinniped pups to factors stimulating myoglobin production, such as submergence-induced hypoxia and exercise training, could explain why muscle oxygen stores take longest to develop. For phocid pups at least, this idea is supported by a link between dependency periods and development of muscle oxygen stores. Hooded seals have a 4-day dependency period, the shortest of any mammal, and these pups rapidly increase myoglobin concentration in their swimming muscles within their first month of life, concurrent with increased muscular activity and early entry into the water (Geiseler et al. 2013). The intersection of these disparate impacts is well-suited for investigation using omic approaches, as this could uncover the timing and relative importance of each driver in the development of the diving phenotype via regulatory gene expression.

Hypoxia

Marine mammals impressively draw down their oxygen stores during diving, tolerating hypoxemia, and cellular hypoxia (Qvist et al. 1986; Meir et al. 2009). Moreover, during submergence, peripheral vasoconstriction prioritizes blood flow and thus oxygen availability to vital organs, but exposes tissues lacking internal oxygen stores to potentially critical levels of ischemia and hypoxia (Zapol et al. 1979). Oxygen delivery is restored at the surface, where reperfusion creates the potential for oxidant production and oxidative stress (Elsner et al. 1998; Zenteno-Savín et al. 2002). Despite life-long exposure to bouts of ischemia and reperfusion, adult seals do not show higher levels of oxidative damage than terrestrial animals, likely the result of high tissue antioxidants (Wilhelm Filho et al. 2002; Vázquez-Medina et al. 2006; Vázquez-Medina et al. 2012). In particular, antioxidant protection from the glutathione system appears central to the diving phenotype (Murphy and Hochachka 1981; Vázquez-Medina et al. 2007). Importantly, glutathione content is increased in the muscles of hooded seals during maturation, along with glutathione peroxidase, glutaredoxin 1, peroxiredoxin IV, thioredoxin 1, and thioredoxin reductase (Vázquez-Medina et al. 2011a). These ontogenic changes in cellular antioxidant status do not appear associated with Nrf2 activation in hooded seals, as the protein and mRNA levels of this transcription factor targeted to antioxidant response elements remain unchanged across maturation (Vázquez-Medina et al. 2011a). Conversely, Nrf2 is significantly increased in response to apneas, as well as across the post-weaning fast in northern elephant seals, which has been tied to induction of antioxidant expression (Vázquez-Medina et al. 2011b; Vázquez-Medina et al. 2013).

Hypoxia-inducible factor (HIF) transcription factor is critical for maintaining cellular oxygen homeostasis and it remains under-studied in freely diving animals. *In utero* hypoxia exposure has been suggested to facilitate the initial development of physiological protective mechanisms. Studies in nondiving species have shown short ischemia/reperfusion intervals can diminish injury in cardiac and skeletal muscle through preconditioning (Murry et al. 1986; Mounsey et al. 1992; Pang and Forrest 1995). This effect may drive the development and maintenance of cell protective mechanisms in pinnipeds. For example, gestating Weddell seals make markedly longer and deeper foraging dives in the weeks prior to birth (Shero et al. 2018); these prolonged dives may initiate fetal and subsequent pup adaptations to hypoxia via preconditioning (Elsner et al. 1969; Hill et al. 1987). Pregnancy is a logistically difficult life history stage to evaluate in pinnipeds; however, developing pups are more accessible to researchers. Vázquez-Medina et al. (2011b) investigated the impact of apnea on oxidative mechanisms in northern elephant seal pup muscle and found that instead of increasing systemic oxidative damage, repeated apneas stimulated oxidative stress protective responses. Northern elephant seals undergo an extended post-weaning fast, during which they experience sleep apneas (Blackwell and Boeuf 1993; Castellini et al. 1994) and increasing time spent submerged in shallow, off-shore waters (Thorson and Le Boeuf 1994). Elephant seal pups may be using short bouts of apnea early in life to precondition their responses to oxidative stress, preparing their tissues for a life of extended hypoxic periods. HIF production is also upregulated over this timeframe in elephant seal pups, which may prepare them for a future of deep-diving and prolonged hypoxia exposure (Soñanez-Organis et al. 2013). The downstream impacts of HIF transcription factor activities on expression of genes with hypoxia-responsive elements will likely provide important clues to the manifestation of the elite diving phenotype.

Muscle fiber type

Muscles contain a distribution of fiber types, each with their own myosin heavy chain isoform signatures and associated metabolic biomarkers including citrate synthase (CS), succinate dehydrogenase, lactate dehydrogenase (LDH), and myoglobin (Zierath and Hawley 2004). The muscles of diving mammals are largely prejudiced toward Type I (slow-twitch oxidative) fiber type profiles (Reed et al. 1994; Watson et al. 2003; Kanatous et al. 2008; Moore et al. 2014), indicating a predisposition for aerobic energy production, despite the potential for vasoconstriction and local hypoxia during submergence (Zapol et al. 1979; Cherepanova et al. 1993; Guyton et al. 1995). Several studies have identified differences in fiber types between pinniped swimming and nonswimming muscles (e.g., Kanatous et al. 2002; Shero et al. 2012), as well as key developmental shifts indicating maturing metabolic profiles (Kanatous et al. 2008; Lestyk et al. 2009; LaRosa et al. 2012; Shero et al. 2012; Moore et al. 2014). Fiber typing has even been applied to indicate degree of maturity in the muscles of pinniped pups at different ages across species (Shero et al. 2019).

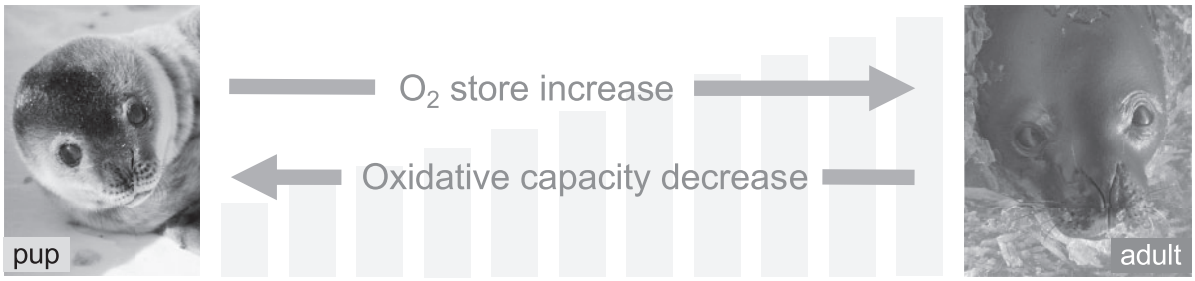
Weddell seal pup muscles have a significantly greater mitochondrial volume density and proportion of Type I fibers than either juveniles or adults (Kanatous et al. 2008). As pups mature, their swimming muscle composition decreases in Type I and increases in Type IIa (fast-twitch oxidative glycolytic) fibers. Weddell seals may be unique in this developmental shift in fiber type in tandem with decreased mitochondrial density. While these findings suggest pups and juveniles uniquely have a higher aerobic potential than adults (Kanatous et al. 2008), this was proposed to reflect thermoregulatory specializations needed to maintain endothermy in a polar environment (Noren et al. 2008). Contrary to the Weddell seal, northern elephant seal swimming muscles become increasingly aerobic during maturation, with Types IIa and IIb myosin heavy chains detectable only in pups (adult muscle fibers become exclusively Type I slow-twitch oxidative, Moore et al. 2014). In an otariid swimming muscle (trapezius in the Australian fur seal), development also includes a shift from Type IIa to Type I fiber profiles (LaRosa et al. 2012).

Shero et al. (2019) investigated the link between duration of the dependency period on the *in utero* development of fiber type profiles in various pinnipeds. Hooded seals, a highly precocial phocid, are born with more mature myosin heavy chain isoform profiles compared to a more altricial phocid, the harp seal. In otariids, the precocial northern fur seal (*Callorhinus ursinus*) is also born with more mature musculature compared to the relatively more altricial Steller sea lion. Importantly, this study suggests that maturation of muscle contractile machinery (and likely metabolic baselines) is prioritized over building muscle oxygen stores in pinniped pups. Developmental priority and sequence of metabolic and physiological phenotypes could be uncovered through investigations into gene regulation.

Metabolic enzymes

Despite detecting a shift in muscle fiber type with maturation (from Type I to IIa), Kanatous et al. (2008) did not note any age-differences in the enzyme activities of CS or cytochrome *c* oxidase in Weddell seals. This indicates a repurposing of metabolic capacity within muscle fiber types, as the lack of enzyme activity differences does not match morphological maturation. One element of submergence capabilities and hypoxia tolerance expected for adult pinnipeds is an ability to extend diving beyond what is fueled by body oxygen stores using glycolytic metabolism (Castellini et al. 1981). As such, increases in anaerobic enzyme capabilities of skeletal and cardiac muscles have been documented with maturation in pinnipeds (Burns et al. 2015). This has been studied in seals (Kanatous et al. 2008; Burns et al. 2010; Prewitt et al. 2010) and sea lions (Shero et al. 2012), primarily by assaying LDH activity as an indicator of anaerobic potential.

Pinniped pups experience diet shifts as they transition to independence. Dramatic changes in thermoregulatory strategy also occur as terrestrial pups, protected by lanugo, undergo molt, and enter the water post-weaning (Noren et al. 2008). Investigations of metabolic enzymes across development have revealed age-differences in anaerobic and oxidative metabolism that may be linked to diet and thermoregulation; however, these shifts are also confounded with metabolic maturation to support hypoxia tolerance (Lestyk et al. 2009; Prewitt et al. 2010). The muscles of Weddell seal pups have enhanced 3-hydroxyacyl-CoA dehydrogenase (HAD) and lipase activity compared to older age classes, indicating a greater dependence on fatty acid metabolism, presumably associated with nursing (Kanatous et al. 2008). Northern fur seal pups rely more on lipid oxidation compared to adults, with higher HAD activities and lower CS: HAD ratios (Shero et al. 2012). In Australian fur seals, adult muscle has significantly higher NADH-tetrazolium reductase (NADH-TR) activity than pups and juveniles



Heme O_2-binding proteins: Hemoglobin, Myoglobin
MicroRNA targets for hematopoiesis: Cd44
MicroRNA targets for iron regulation: Tfrc, Ireb2

Mitochondrial (Mt) oxidative capacity: Volume density
MicroRNA targets for Mt respiration: Cox4i1, Cox10, Ndufs7, Ndor1, Sdhaf2
Muscle fiber Type I to IIa: Calcineurin, SERCA2
MicroRNA targets for calcineurin signaling: Rcan1, Rcan2, Cabin1
MicroRNA targets for Ca-ATPases: ATP1a3, ATP2b2, ATP2b3, ATP2b4

Fig. 1 Protein biomarkers of Weddell seal physiological maturation include elaboration of oxygen binding proteins and alterations in muscle fiber type and mitochondrial volume density to decrease oxidative capacity (Kanatous et al. 2008). The mechanism driving these developments is likely gene regulation, which to date has only been explicitly explored in a miRNA sequencing study (Penso Dolfin et al. 2020). This figure overlays complementary elements of these datasets (proteins reported in Kanatous et al. 2008 in bold, predicted mRNA targets of miRNAs reported in Penso Dolfin et al. 2020 that differ consistently by age class in italics), to highlight future possibilities to investigate development of the diving phenotype in this species.

(LaRosa et al. 2012). Further investigation of metabolic enzymes overlaid with HIF signaling will be important in distinguishing phenotypic regulation associated with diet and thermoregulatory specializations from metabolic development toward a mature diver.

An example of gene regulation studies: microRNAs in the Weddell seal

The Weddell seal is an excellent candidate to apply single-species physiological, biochemical, and genomic approaches to tease apart the basis of extreme diving physiology by examining development. Simple physiological studies are insufficient, as this species displays a clear overlap between dietary and thermoregulatory development on the same time scale as maturation of the diving phenotype. Still, the baseline physiology and ontogeny of this species are extremely well-understood, and an annotated genome is available.

To discuss a role for gene regulation in development of the mature phenotype, we consider the overlap of miRNA sequencing study in Weddell seals on the known physiological and biochemical features involved in the development of the diving phenotype. Differentially expressed miRNAs between adults and pups (Penso Dolfin et al. 2020) target gene regulation pathways consistent with common and species-specific ontogeny. As with other pinnipeds, Weddell seal pups have limited oxygen stores relative to adults; miRNAs differentially expressed across development target mRNAs associated with hematopoiesis and iron regulatory signaling (Fig. 1). Weddell seal pups additionally display two unique features of skeletal muscle development; a shift away from oxidative Type I fibers, and higher mitochondrial volume densities, likely to enhance nonshivering thermogenesis (Kanatous et al. 2008; Noren et al. 2008). This ontogenic shift includes changes in markers of calcium signaling and handling (e.g., calcineurin B, SERCA2) proposed to regulate skeletal muscle fiber conversion via the calcium/NFAT pathway (Kanatous et al. 2008). Similarly, miRNAs with developmentally distinct expression levels target genes associated with calcium signaling/muscle fiber types and mitochondrial respiratory capacity (Fig. 1). Furthermore, previously unannotated, Weddell-specific miRNAs also differ between pups and adults. Novel miRNAs are acknowledged as an early evolutionary mechanism controlling species-specific gene regulation (Meunier et al. 2013), versus previously annotated miRNAs more likely to regulate typical mammalian developmental processes. Novel miRNAs are therefore excellent candidates to drive the maturation of the elite diving phenotype (Penso Dolfin et al. 2020). Subsequent functional studies of gene regulation may allow us to tease apart diet, thermoregulation, and diving pressures to identify mechanistic drivers that build a hypoxia tolerant phenotype in developing pinnipeds.

Additional single-species omics approaches may yield novel insights into the genetic underpinnings of diving physiology. This could include studies of the timing of gene expression across ontogeny compared against development of diving capabilities to link genome to phenotype. In particular, what is missing from our knowledge of diving physiology

and biochemistry is an understanding of the metabolic and cellular protective status necessary to tolerate submergence hypoxia. In our example, Penso Dolfin et al. (2020) sequenced plasma from healthy animals, as well as the brain, heart, and muscle from necropsies. Functional genomic studies that reveal the time course and transcription factor capabilities of HIF1α in tissues will establish mechanistic links between environmental stressors and the manifestation of phenotype. Metabolomic profiles from post-dive blood samples will also confirm metabolic fuel use across different dive types and the manifestation or avoidance of cellular stress during diving.

Conclusions

This review addresses the development of diving physiology in pinnipeds, which are unique among marine mammals in their transition from terrestrial newborns to novice-diving juveniles to elite-diving adults. This maturation primarily occurs in body oxygen stores and metabolic pathways, but also includes cell protective mechanisms that may be a key to hypoxia tolerance. While there is significant literature that describes the ontogeny of diving physiology in pinnipeds, only limited studies address mechanisms that offer insights into the underlying molecular and genetic elements of this extreme phenotype. We suggest that a developmental viewpoint may advance our mechanistic understanding of comparative physiology by facilitating investigation of extreme phenotypes within a single species with a modern molecular and genomic toolkit.

A challenge of this approach is that it requires parsing the regulation of ontogeny from the overall phenotype. For example, the development of pinniped pups to independence includes alterations in diet, thermoregulatory strategy, and diving competence, and it can be difficult to tease out the mechanistic drivers of hypoxia tolerance simply via protein biomarkers. Exploring the genomic and molecular underpinnings using an ontogeny framework is the next step toward understanding the maturation of the diving phenotype, general pinniped development, and how they overlap.

References

Blackwell SB, Boeuf BL. 1993. Developmental aspects of sleep apnoea in northern elephant seals, *Mirounga angustirostris*. J Zool 231:437–47.

Boaz SM, Champagne CD, Fowler MA, Houser DH, Crocker DE. 2012. Water-soluble vitamin homeostasis in fasting northern elephant seals (*Mirounga angustirostris*) measured by metabolomics analysis and standard methods. Comp Biochem Physiol A Mol Integr Physiol 161:114–21.

Burns J, Castellini M. 1996. Physiological and behavioral determinants of the aerobic dive limit in Weddell seal (*Leptonychotes weddellii*) pups. J Comp Physiol B 166:473–83.

Burns J, Costa D, Frost K, Harvey J. 2005. Development of body oxygen stores in harbor seals: effects of age, mass, and body composition. Physiol Biochem Zool 78:1057–68.

Burns J, Hammill M. 2008. Does iron availability limit oxygen store development in seal pups. Proceedings of the 4th CPB Meeting in Africa: MARA 2008 "Molecules to Migration: The Pressures of Life." Bologna, Italy: Medimond International Proceedings.

Burns J, Lestyk K, Folkow L, Hammill M, Blix A. 2007. Size and distribution of oxygen stores in harp and hooded seals from birth to maturity. J Comp Physiol B 177:687–700.

Burns J, Lestyk K, Freistroffer D, Hammill M. 2015. Preparing muscles for diving: age-related changes in muscle metabolic profiles in harp (*Pagophilus groenlandicus*) and hooded (*Cystophora cristata*) seals. Physiol Biochem Zool 88:167–82.

Burns J, Skomp N, Bishop N, Lestyk K, Hammill M. 2010. Development of aerobic and anaerobic metabolism in cardiac and skeletal muscles from harp and hooded seals. J Exp Biol 213:740–8.

Burns JM. 1999. The development of diving behavior in juvenile Weddell seals: pushing physiological limits in order to survive. Can J Zool 77:737–47.

Burns JM, Clark CA, Richmond JP. 2004. The impact of lactation strategy on physiological development of juvenile marine mammals: implications for the transition to independent foraging. Int Congr Ser 1275:341–50.

Calvete JJ, Sanz L, Cid P, de la Torre P, Flores-Díaz M, Dos Santos MC, Borges A, Bremo A, Angulo Y, Lomonte B, et al. 2010. Snake venomics of the Central American rattlesnake *Crotalus simus* and the South American *Crotalus durissus* complex points to neurotoxicity as an adaptive Paedomorphic trend along Crotalus dispersal in South America. J Proteome Res 9:528–44.

Castellini MA, Milsom WK, Berger RJ, Costa DP, Jones DR, Castellini JM, Rea LD, Bharma S, Harris M. 1994. Patterns of respiration and heart rate during wakefulness and sleep in elephant seal pups. Am J Physiol 266:R863–R869.

Castellini MA, Somero GN, Kooyman GL. 1981. Glycolytic enzyme activities in tissues of marine and terrestrial mammals. Physiol Zool 54:242–52.

Champagne CD, Boaz SM, Fowler MA, Houser DS, Costa DP, Crocker DE. 2013. A profile of carbohydrate metabolites in the fasting northern elephant seal. Comp Biochem Physiol Part D Genom Proteom 8:141–51.

Cherepanova V, Neshumova T, Elsner R. 1993. Muscle blood flow in diving mammals. Comp Biochem Physiol A Mol Integr Physiol 106:1–6.

Chikina M, Robinson JD, Clark NL. 2016. Hundreds of genes experienced convergent shifts in selective pressure in marine mammals. Mol Biol Evol 33:2182–92.

Clark CA, Burns JM, Schreer JF, Hammill MO. 2006. Erythropoietin concentration in developing harbor seals (*Phoca vitulina*). Gen Comp Endocrinol 147:262–7.

Clark CA, Burns JM, Schreer JF, Hammill MO. 2007. A longitudinal and cross-sectional analysis of total body oxygen

store development in nursing harbor seals (*Phoca vitulina*). J Comp Physiol B 177:217–27.

Darbellay F, Necsulea A. 2020. Comparative transcriptomics analyses across species, organs, and developmental stages reveal functionally constrained incrnas. Mol Biol Evol 37:240–59.

De Miranda MA Jr, Schlater AE, Green TL, Kanatous SB. 2012. In the face of hypoxia: myoglobin increases in response to hypoxic conditions and lipid supplementation in cultured Weddell seal skeletal muscle cells. J Exp Biol 215:806–13.

Dinkelacker S, Costanzo J, Lee R. 2005. Anoxia tolerance and freeze tolerance in hatchling turtles. J Comp Physiol B 175:209–17.

Durban J, Pérez A, Sanz L, Gómez A, Bonilla F, Rodríguez S, Chacón D, Sasa M, Angulo Y, Gutiérrez JM, et al. 2013. Integrated "omics" profiling indicates that miRNAs are modulators of the ontogenetic venom composition shift in the Central American rattlesnake, *Crotalus simus* simus. BMC Genomics 14:234.

Elsner R, Hammond D, Parker H. 1969. Circulatory responses to asphyxia in pregnant and fetal animals: a comparative study of Weddell seals and sheep. Yale J Biol Med 42:202–17.

Elsner R, Øyasæter S, Almaas R, Saugstad OD. 1998. Diving seals, ischemia-reperfusion and oxygen radicals. Comp Biochem Physiol A Mol Integr Physiol 119:975–80.

Fanter CE, Lin Z, Keenan SW, Janzen FJ, Mitchell TS, Warren DE. 2020. Development-specific transcriptomic profiling suggests new mechanisms for anoxic survival in the ventricle of overwintering turtles. J Exp Biol 223:jeb213918.

Foote AD, Liu Y, Thomas GWC, Vinař T, Alföldi J, Deng J, Dugan S, van Elk CE, Hunter ME, Joshi V, et al. 2015. Convergent evolution of the genomes of marine mammals. Nat Genet 47:272–5.

Fowler S, Costa D, Arnould J, Gales N, Burns J. 2007. Ontogeny of oxygen stores and physiological diving capability in Australian sea lions. Funct Ecol 21:922–35.

Geiseler SJ, Blix AS, Burns JM, Folkow LP. 2013. Rapid postnatal development of myoglobin from large liver iron stores in hooded seals. J Exp Biol 216:1793–8.

Greaves DK, Hughson RL, Topor Z, Schreer JF, Burns JM, Hammill MO. 2004. Changes in heart rate variability during diving in young harbor seals, *Phoca vitulina*. Mar Mamm Sci 20:861–71.

Gutiérrez JM, Dos Santos MC, de Fatima Furtado M, Rojas G. 1991. Biochemical and pharmacological similarities between the venoms of newborn *Crotalus durissus* durissus and adult *Crotalus durissus* Terrificus rattlesnakes. Toxicon 29:1273–7.

Guyton GP, Stanek KS, Schneider RC, Hochachka PW, Hurford WE, Zapol DG, Liggins GC, Zapol WM. 1995. Myoglobin saturation in free-diving Weddell seals. J Appl Physiol 79:1148–55.

Hao Y, Xiong Y, Cheng Y, Song G, Jia C, Qu Y, Lei F. 2019. Comparative transcriptomics of 3 high-altitude passerine birds and their low-altitude relatives. Proc Natl Acad Sci U S A 116:11851–6.

Hill RD, Schneider RC, Liggins GC, Schuette AH, Elliott RL, Guppy M, Hochachka PW, Qvist J, Falke KJ, Zapol WM. 1987. Heart rate and body temperature during free diving of Weddell seals. Am J Physiol 253:R344–351.

Kanatous S, Davis R, Watson R, Polasek L, Williams T, Mathieu-Costello O. 2002. Aerobic capacities in the skeletal muscles of Weddell seals: key to longer dive durations? J Exp Biol 205:3601–8.

Kanatous S, Hawke T, Trumble S, Pearson L, Watson R, Garry D, Williams T, Davis R. 2008. The ontogeny of aerobic and diving capacity in the skeletal muscles of Weddell seals. J Exp Biol 211:2559–65.

Khudyakov J, Champagne C, Meneghetti L, Crocker D. 2017. Blubber transcriptome response to acute stress axis activation involves transient changes in Adipogenesis and lipolysis in a fasting-adapted marine mammal. Sci Rep 7:42110.

Khudyakov JI, Preeyanon L, Champagne CD, Ortiz RM, Crocker DE. 2015. Transcriptome analysis of northern elephant seal (*Mirounga angustirostris*) muscle tissue provides a novel molecular resource and physiological insights. BMC Genomics 16:64.

LaRosa DA, Cannata DJ, Arnould JPY, O'Sullivan LA, Snow RJ, West JM. 2012. Changes in muscle composition during the development of diving ability in the Australian fur seal. Aust J Zool 60:81–90.

Lestyk KC, Folkow L, Blix A, Hammill M, Burns J. 2009. Development of myoglobin concentration and acid buffering capacity in harp (*Pagophilus groenlandicus*) and hooded (*Cystophora cristata*) seals from birth to maturity. J Comp Physiol B 179:985–96.

MacArthur RA. 1990. Seasonal changes in the oxygen storage capacity and aerobic dive limits of the muskrat (*Ondatra zibethicus*). J Comp Physiol B 160:593–9.

MacArthur RA, Humphries M, Fines G, Campbell K. 2001. Body oxygen stores, aerobic dive limits, and the diving abilities of juvenile and adult muskrats (*Ondatra zibethicus*). Physiol Biochem Zool 74:178–90.

Meir JU, Champagne CD, Costa DP, Williams CL, Ponganis PJ. 2009. Extreme hypoxemic tolerance and blood oxygen depletion in diving elephant seals. Am J Physiol Regul Integr Comp Physiol 297:R927–R939.

Meunier J, Lemoine F, Soumillon M, Liechti A, Weier M, Guschanski K, Hu H, Khaitovich P, Kaessmann H. 2013. Birth and expression evolution of mammalian microRNA genes. Genom Res 23:34–45.

Mirceta S, Signore AV, Burns JM, Cossins AR, Campbell KL, Berenbrink M. 2013. Evolution of mammalian diving capacity traced by myoglobin net surface charge. Science 340:1234192.

Moore CD, Crocker DE, Fahlman A, Moore MJ, Willoughby DS, Robbins KA, Kanatous SB, Trumble SJ. 2014. Ontogenetic changes in skeletal muscle fiber type, fiber diameter and myoglobin concentration in the northern elephant seal (*Mirounga angustirostris*). Front Physiol 5:217.

Morrison P, Rosenmann M, Sealander JA. 1966. Seasonal variation of myoglobin in the northern red-backed vole. Am J Physiol 211:1305–8.

Mounsey R, Pang C, Boyd J, Forrest C. 1992. Augmentation of skeletal muscle survival in the latissimus dorsi porcine model using acute ischemic preconditioning. J Otolaryngol 21:315–20.

Murphy BJ, Hochachka PW. 1981. Free amino acid profiles in blood during diving and recovery in the Antarctic Weddell seal. Can J Zool 59:455–9.

Murry CE, Jennings RB, Reimer KA. 1986. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 74:1124–36.

Noren SR, Edwards MS. 2020. Mature hematology in neonatal walruses supports diving alongside their mothers. Mar Mamm Sci 36:595–611.

Noren SR, Iverson SJ, Boness DJ. 2005. Development of the blood and muscle oxygen stores in gray seals (*Halichoerus grypus*): implications for juvenile diving capacity and the necessity of a terrestrial postweaning fast. Physiol Biochem Zool 78:482–90.

Noren SR, Pearson LE, Davis J, Trumble SJ, Kanatous SB. 2008. Different thermoregulatory strategies in nearly weaned pup, yearling, and adult Weddell seals (*Leptonychotes weddelli*). Physiol Biochem Zool 81:868–79.

Odegard DT, Sonnenfelt MA, Bledsoe JG, Keenan SW, Hill CA, Warren DE. 2018. Changes in the material properties of the shell during simulated aquatic hibernation in the anoxia-tolerant painted turtle. J Exp Biol 221:jeb176990.

Pang CY, Forrest CR. 1995. Acute pharmacologic preconditioning as a new concept and alternative approach for prevention of skeletal muscle ischemic necrosis. Biochem Pharmacol 49:1023–34.

Penso Dolfin L, Haerty W, Hindle A, Di Palma F. 2020. MicroRNA profiling in the Weddell seal suggests novel regulatory mechanisms contributing to diving adaptation. BMC Genomics 21:17.

Pervolaraki E, Dachtler J, Anderson RA, Holden AV. 2018. The developmental transcriptome of the human heart. Sci Rep 8:1–9.

Podrabsky JE, Carpenter JF, Hand SC. 2001. Survival of water stress in annual fish embryos: dehydration avoidance and egg envelope amyloid fibers. Am J Physiol Regul Integr Comp Physiol 280:R123–R131.

Podrabsky JE, Hrbek T, Hand SC. 1997. Physical and chemical characteristics of ephemeral pond habitats in the Maracaibo basin and llanos region of Venezuela. Hydrobiologia 362:67–77.

Ponganis PJ, Meir JU, Williams CL. 2011. In pursuit of Irving and Scholander: a review of oxygen store management in seals and penguins. J Exp Biol 214:3325–39.

Prewitt J, Freistroffer D, Schreer J, Hammill M, Burns J. 2010. Postnatal development of muscle biochemistry in nursing harbor seal (*Phoca vitulina*) pups: limitations to diving behavior? J Comp Physiol B 180:757–66.

Qvist J, Hill RD, Schneider RC, Falke KJ, Liggins GC, Guppy M, Elliot RL, Hochachka PW, Zapol WM. 1986. Hemoglobin concentrations and blood gas tensions of free-diving Weddell seals. J Appl Physiol 61:1560–9.

Reed J, Butler P, Fedak M. 1994. The metabolic characteristics of the locomotory muscles of grey seals (*Halichoerus grypus*), harbour seals (*Phoca vitulina*) and Antarctic fur seals (*Arctocephalus gazella*). J Exp Biol 194:33–46.

Reese SA, Ultsch GR, Jackson DC. 2004. Lactate accumulation, glycogen depletion, and shell composition of hatchling turtles during simulated aquatic hibernation. J Exp Biol 207:2889–95.

Richmond JP, Burns JM, Rea LD. 2006. Ontogeny of total body oxygen stores and aerobic dive potential in steller sea lions (*Eumetopias jubatus*). J Comp Physiol B 176:535–45.

Riggs CL, Podrabsky JE. 2017. Small noncoding RNA expression during extreme anoxia tolerance of annual killifish (*Austrofundulus limnaeus*) embryos. Physiol Genomics 49:505–18.

Riggs CL, Summers A, Warren DE, Nilsson GE, Lefevre S, Dowd WW, Milton S, Podrabsky JE. 2018. Small noncoding RNA expression and vertebrate anoxia tolerance. Front Genet 9:230.

Romney AL, Davis EM, Corona MM, Wagner JT, Podrabsky JE. 2018. Temperature-dependent vitamin d signaling regulates developmental trajectory associated with diapause in an annual killifish. Proc Natl Acad Sci U S A 115:12763–8.

Saunders D, Fedde M. 1991. Physical conditioning: effect on the myoglobin concentration in skeletal and cardiac muscle of bar-headed geese. Comp Biochem Physiol, A Mol Integr Physiol 100:349–52.

Shaffer BH, Minx P, Warren DE, Shedlock AM, Thomson RC, Valenzuela N, Abramyan J, Amemiya CT, Badenhorst D, Biggar KK, et al. 2013. The western painted turtle genome, a model for the evolution of extreme physiological adaptations in a slowly evolving lineage. Genome Biol 14:R28.

Shero MR, Andrews RD, Lestyk KC, Burns JM. 2012. Development of the aerobic dive limit and muscular efficiency in northern fur seals (*Callorhinus ursinus*). J Comp Physiol B 182:425–36.

Shero MR, Goetz KT, Costa DP, Burns JM. 2018. Temporal changes in Weddell seal dive behavior over winter: are females increasing foraging effort to support gestation? Ecol Evol 8:11857–74.

Shero MR, Reiser PJ, Simonitis L, Burns JM. 2019. Links between muscle phenotype and life history: differentiation of myosin heavy chain composition and muscle biochemistry in Precocial and Altricial pinniped pups. J Comp Physiol B 189:717–34.

Soñanez-Organis JG, Vázquez-Medina JP, Crocker DE, Ortiz RM. 2013. Prolonged fasting activates hypoxia inducible factors-1α,-2α and-3α in a tissue-specific manner in northern elephant seal pups. Gene 526:155–63.

Spence-Bailey L, Verrier D, Arnould J. 2007. The physiological and behavioural development of diving in Australian fur seal (*Arctocephalus pusillus* doriferus) pups. J Comp Physiol B 177:483–94.

Stephenson R, Turner D, Butler P. 1989. The relationship between diving activity and oxygen storage capacity in the tufted duck (*Aythya fuligula*). J Exp Biol 141:265–75.

Storey KB, Storey JM, Brooks S, Churchill TA, Brooks RJ. 1988. Hatchling turtles survive freezing during winter hibernation. Proc Natl Acad Sci U S A 85:8350–4.

Thomas GWC, Hahn MW, Hahn Y. 2017. The effects of increasing the number of taxa on inferences of molecular convergence. Genome Biol Evol 9:213–21.

Thorson PH, Le Boeuf B. 1994. Developmental aspects of diving in northern elephant seal pups. Elephant seals: population ecology, behavior, and physiology. Berkeley (CA): University of California Press. p. 271–89.

Toni LS, Padilla PA. 2016. Developmentally arrested *Austrofundulus limnaeus* embryos have changes in post-

translational modifications of histone h3. J Exp Biol 219:544–52.
Ultsch GR. 1989. Ecology and physiology of hibernation and overwintering among freshwater fishes, turtles, and snakes. Biol Rev 64:435–515.
Vázquez-Medina JP, Soñanez-Organis JG, Burns JM, Zenteno-Savín T, Ortiz RM. 2011a. Antioxidant capacity develops with maturation in the deep-diving hooded seal. J Exp Biol 214:2903–10.
Vázquez-Medina JP, Soñanez-Organis JG, Rodriguez R, Viscarra JA, Nishiyama A, Crocker DE, Ortiz RM. 2013. Prolonged fasting activates nrf2 in post-weaned elephant seals. J Exp Biol 216:2870–8.
Vázquez-Medina JP, Zenteno-Savin T, Elsner R. 2006. Antioxidant enzymes in ringed seal tissues: potential protection against dive-associated ischemia/reperfusion. Comp Biochem Physiol C Toxicol Pharmacol 142:198–204.
Vázquez-Medina JP, Zenteno-Savín T, Elsner R. 2007. Glutathione protection against dive-associated ischemia/reperfusion in ringed seal tissues. J Exp Mar Biol Ecol 345:110–8.
Vázquez-Medina JP, Zenteno-Savin T, Elsner R, Ortiz RM. 2012. Coping with physiological oxidative stress: a review of antioxidant strategies in seals. J Comp Physiol B 182:741–50.
Vázquez-Medina JP, Zenteno-Savín T, Tift MS, Forman HJ, Crocker DE, Ortiz RM. 2011b. Apnea stimulates the adaptive response to oxidative stress in elephant seal pups. J Exp Biol 214:4193–200.
Verrier D, Guinet C, Authier M, Tremblay Y, Shaffer S, Costa DP, Groscolas R, Arnould JP. 2011. The ontogeny of diving abilities in subantarctic fur seal pups: developmental tradeoff in response to extreme fasting? Funct Ecol 25:818.
Wagner JT, Singh PP, Romney AL, Riggs CL, Minx P, Woll SC, Roush J, Warren WC, Brunet A, Podrabsky JE. 2018. The genome of *Austrofundulus limnaeus* offers insights into extreme vertebrate stress tolerance and embryonic development. BMC Genomics 19:155.
Watson RR, Miller TA, Davis RW. 2003. Immunohistochemical fiber typing of harbor seal skeletal muscle. J Exp Biol 206:4105–11.
Weise MJ, Costa DP. 2007. Total body oxygen stores and physiological diving capacity of California sea lions as a function of sex and age. J Exp Biol 210:278–89.
Wilhelm Filho D, Sell F, Ribeiro L, Ghislandi M, Carrasquedo F, Fraga C, Wallauer J, Simões-Lopes P, Uhart M. 2002. Comparison between the antioxidant status of terrestrial and diving mammals. Comp Biochem Physiol A Mol Integr Physiol 133:885–92.
Wourms JP. 1972. The developmental biology of annual fishes. Iii. Pre-embryonic and embryonic diapause of variable duration in the eggs of annual fishes. J Exp Zool 182:389.
Zapol WM, Liggins G, Schneider R, Qvist J, Snider MT, Creasy RK, Hochachka PW. 1979. Regional blood flow during simulated diving in the conscious Weddell seal. J Appl Physiol 47:968–73.
Zenteno-Savín T, Clayton-Hernández E, Elsner R. 2002. Diving seals: are they a model for coping with oxidative stress? Comp Biochem Physiol C Toxicol Pharmacol 133:527–36.
Zierath JR, Hawley JA. 2004. Skeletal muscle fiber type: influence on contractile and metabolic properties. PLoS Biol 2:e348.