HAND-REARING HARBOUR SEAL PUPS (*PHOCA VITULINA*): THE EFFECT OF DIET AND SUPPLEMENTARY HEAT ON GROWTH AND SURVIVAL

by

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Abstract

Hundreds of stranded harbour seals pups (*Phoca vitulina*) are brought to wildlife rescue centres every year, often unweaned and in poor body condition. Typical hand-rearing diets include artificial milk-replacers and diets based on macerated fish, both normally fed via gavage. Mortality rates for these animals can be high and weight gains on artificial formulas are low. This study was designed to determine the effect of the following treatments on the growth and survival of captive orphaned seals: (1) feeding pups an artificial milk-replacer versus a fish-based formula; and (2) the provision of supplementary heat. Pups admitted to the facility in summer 2007 (n=145) and 2008 (n=98) were randomly assigned to one of two diets and fed until weaning at roughly 20 days of age. In 2008, 25 pups were also provided with a supplementary heat source. Diet and heat treatments were compared using average daily gain (ADG) and mortality rates. In 2007, with pups fed formulas at 8% of body weight per day, pups fed milk-replacer gained more (43 g/d ± 12, mean ± SEM) than those fed fish-formula (loss of 13 g/d ± 6; $p<0.01$) and their survival to weaning was twice as high ($p<0.05$, chi-squared analysis). In 2008, with daily intake increased to 11% of body weight per day, weight gain was improved for both diets but remained higher on milk-replacer (123 g/d ± 12, loss of 6 g/day ± 8; $p<0.01$) and fewer seals died on either treatment (6/35 on milk replacer, 8/34 on fish formula.) Pups provided with heat did not show any differences in ADG ($p>0.05$) or survival rate ($p>0.05$) but did show increased heat-seeking behaviour at ambient temperatures below 16°C ($p<0.001$). I conclude: (1) although neither diet achieved the weight gains recorded in mother-raised pups (400 – 800 g/d), the artificial milk formula was clearly more successful; (2) supplementary heat can be safely used for unweaned pups in poor body condition at ambient temperatures <16°C; and (3) more work is needed on both diet composition and feeding method to achieve higher survival and more natural weight gains.

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Co-authorship statement

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Chapter 1: General introduction

1.1 Population status

The harbour seal subspecies *Phoca vitulina ricardsi* is widely distributed in the eastern Pacific Ocean, ranging along the coast from California to Alaska (Shaughnessy and Fay 1977; Olseuik 1999). In British Columbia, close to half a million harbour seals are estimated to have been killed for pelts and predator bounty payments since the late 1870s (Bigg 1969; Boulva and Mclaren 1979; Olseuik et al 1990). This over-hunting reduced the population below sustainable levels, and by the 1970s the British Columbian population was estimated to be only 10,000 animals (Olseuik 1999). Since culling ended in 1969, the population has rebounded, growing approximately 12.5% per year between 1973 and 1988 (Olseuik 1990). In 2008, the population had stabilized at close to 100,000 seals, which is thought to be close to original numbers (Olseuik 1999).

The British Columbian population is about 29% of the total harbour seal population in the Northeast Pacific Ocean. Along British Columbia's 27,200 km coastline, the highest densities of harbour seals are found in the Straight of Georgia with an average of 13.1 seals per km of shoreline as compared to 2.6 seals per km in other areas of British Columbia. This means that 37% of the British Columbian harbour seal population inhabits just 11% of the coastline (Olseuik 1999). This concentrated population is in an area heavy with marine traffic, coastline development and human density. Pups in particular are vulnerable to disturbance as they are born, and primarily nursed, on land (Bowen 1991; Lawson and Renouf 1985).

1.2 Natural history

Harbour seals (*Phoca vitulina*) are mid-size phocids, approximately 1.7 -1.9 m in length and 60 - 150 kg in weight as adults (Burns 2002). Females give birth once per year to precocial, relatively large pups that are 12 -14% of maternal body weight (Bowen 1991; Bowen et al. 1992). Pups weigh between 8 and 12 kg at birth (Burns 2002; Cottrell *et al.* 2002), and are typically born between mid May and early July in northern British Columbia, and between early July and late August on the southern coast of the province (Temte *et al.* 1991).

Females have a relatively short (24 – 32 day) lactation period during which they rapidly transfer energy to pups in the form of energy-dense, fat-rich milk (Lang *et al.* 2005). Pups gain 400 – 800 g/day (Cottrell et al. 2002; Bowen et al. 1994), more than doubling their natal weight before weaning (Bowen *et al.* 1992; Bowen *et al.* 2001). The majority of research on lactation length and pup weight gain are based on the Atlantic subspecies *Phoca vitulina concolor*. Pups of this subspecies tend to gain weight more rapidly (600-800 g/day) over a shorter lactation period (24 days; Bowen *et al.* 1994) than the British Columbian *Phoca vitulina ricardsi,* which gain closer to 400 g/day during a 32-day lactation period (Cottrell *et al.* 2002). The lower weight gain of *Phoca vitulina ricardsi* possibly is due to the topography of pupping and haul-out sites in British Columbia, where pups are forced to regularly swim and thus expend more energy (Cottrell *et al.* 2002). There is no difference in birth- or weaning-weights between animals in the separate regions, and it is likely that the longer lactation of animals in British Columbia compensates for their slower rate of weight gain (Cottrell *et al.* 2002). In both subspecies the deposition of blubber accounts for the majority of this weight gain; pups have 11% body fat at birth and 39 - 42% body fat at weaning (Muelbert *et al.* 2003). Pups are weaned at approximately 23 – 25 kg (Cottrell *et al.* 2002; Bowen *et al.* 2001).

Many young mammals begin to forage while still nursing, but harbour seal pups' sole source of nutrition until weaning is milk (Bowen 1991). Lactation is terminated when pups are abruptly weaned and left to begin eating solid food without parental assistance (Bonner 1984; Oftedal *et al.* 1987). Pups begin eating within a few days of weaning, but it takes an average of three weeks before they attain a positive energy balance (Muelbert and Bowen 1993; Muelbert *et al.* 2003). During this transition pups rely on their blubber store, using over half of their body fat (and losing 21% of weaning weight) in the first 4-6 weeks post-weaning (Muelbert and Bowen 1993). Body size and the amount of stored energy at weaning are important for the pups' survival (Muelbert and Bowen 1993). Annual mortality estimates for juvenile harbour seals can be very high (35-80%), and vary depending on region (Van Bemmel 1956; Bigg 1969; Reijnders 1978; Reijnders *et al.* 1997; Steiger et al. 1989). However, there is evidence that pups heavier at weaning have a greater chance of surviving their first year than do lighter ones (Harding *et al.* 2005).

1.3 Rehabilitation

Several hundred pups are brought to wildlife rescue facilities in British Columbia every year. For example, the Vancouver Aquarium Marine Mammal Rescue Centre is one of two centres in the province that handles harbour seals. Since 2005, this Centre has admitted between 96 and 174 pups per year. The large majority of these animals were unweaned and in poor body condition.

One of the challenges in raising orphaned harbour seal pups is finding a diet and feeding regime that will give satisfactory weight gains. When released, pups must rely on their fat stores while they learn to locate and capture prey. Therefore, a diet that enables rapid growth in captive care may give rehabilitated animals the body reserves they need to survive after release.

The unique properties of seal milk, especially its very high fat content (Lang *et al.* 2002), make it difficult to replace with hand-rearing formulas. Common hand-rearing diets include artificial milk-replacers and fish-based formulas (Smith and Stone 1992; Townsend and Gage 2001; Lockwood 2004). Feeding rates and recipes vary among rehabilitation facilities, but pup weight gains on these diets are low compared to those of mother-raised pups. For example, 50 g/day gains when fed a fish formula (Wilson *et al.* 1999) and 120-300 g/day gains when fed a milk-replacer (Duerr 2002) have been reported. In another study, pups gained more weight once weaned onto whole herring than when fed formula (Lockwood 2004). Formulas are usually fed via gavage to facilitate the feeding of numerous animals, and to ensure the delivery of apportioned food volumes to each individual. Despite extensive use of such diets, there has been little systematic comparison of their effect on weight gain and mortality of pups.

A second challenge is creating a suitable thermal environment. In the wild, pups would spend much of their time exposed to the elements on the coastline, but their growing layer of blubber, plus the heat generated by digesting large amounts of milk, appears to provide a degree of protection against chilling. However, orphaned seals with poor body condition and slow weight gain may be much more vulnerable. Supplementary heat is successfully used in rearing many domestic species, and has been shown to help promote animals' growth and survival (Curtis 1983). Hence, although supplementary heat is not natural for harbour seal pups in the wild, it is possible that pups undergoing rehabilitation could benefit from a supplementary heat source.

1.4 Objectives

The objectives of this study were: (1) to compare the effect of two commonly used diets – an artificial milk-replacer and a fish-based formula – on the survival and weight gain of orphaned seal pups in captivity, and; (2) to examine whether animals provided with a supplementary heat source would use it in a seemingly adaptive manner, and whether or not they would have greater weight gains and higher survival rates until weaning than animals without supplementary heat.

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Chapter 2: The effect of diet and feeding level on survival and weight gain of hand-raised harbour seal pups (*Phoca vitulina*)[1]

2.1. Introduction

The harbour seal population in British Columbia (*Phoca vitulina richardsi*) is estimated to be over 100,000, 37% of which are concentrated in the Georgia Straight (Olesiuk 1999). In southern British Columbia the pupping season is in July and August (Temte *et al.* 1991), which corresponds with a time of high beach use by humans. Because pups are born, and primarily nursed, on land (Bowen 1991; Lawson and Renouf 1985), they may be vulnerable to disturbance from shoreline development and coastal traffic. Consequently, many pups are abandoned during this period, often because of human activity. As a result, hundreds of orphaned and injured neonatal harbour seals are brought to wildlife rescue centres every summer (Lander *et al.* 2002).

The rehabilitation of orphaned or injured wildlife is controversial. A common argument against such practice is that it does not contribute to the conservation of wild populations, and may actually threaten wild populations, as released individuals may carry disease, introduce novel pathogens, or perpetuate inferior genes (Measures 2004). Another concern is that a focus on the rehabilitation of individuals diverts attention and resources away from larger conservation problems (Fraser and Moss 1985). Nonetheless, public interest in the rescue of abandoned and injured animals is very strong (Kirkwood and Sainsbury 1996), and some facilities working with marine mammals garner public support for their broader work by rescuing and raising orphaned animals. Moreover, knowledge gained in the successful rehabilitation of abundant species can

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play a crucial role in maintaining or rehabilitating rare species. For example, the development, over many years, of veterinary therapies and expertise in the care of more common pinniped species has been applied to the rehabilitation of highly endangered Hawaiian monk seals (*Monachus schauinslandi)* (Moore *et al.* 2007).

Rehabilitation of harbour seal pups presents significant challenges. Pups admitted to wildlife rehabilitation facilities are often emaciated, dehydrated, and have illnesses or wounds requiring treatment (Colegrove *et al.* 2005). Successful rehabilitation is additionally challenged by the fact that most pups are not yet weaned and therefore require a specialized diet to replace energy-rich seal milk until they are ready to eat whole fish.

Rehabilitation facilities use various diets for young seals, with the most common diets based on artificial milk-replacers or macerated fish (Smith and Stone 1992; Townsend and Gage 2001; Lockwood 2004). These formulas are usually fed via gavage to facilitate feeding numerous animals and to ensure that adequate food volumes are delivered to each individual. Despite extensive use of such diets, there has been little systematic comparison of their effects. The purpose of this study was to determine the effects of feeding an artificial milk-replacer or a fish-based formula on the survival and weight gain of orphaned seal pups in captivity. The study also provided a comparison of two different feeding levels used in different years.

2.2. Materials and methods

Harbour seal pups included in this trial were recovered along the British Columbian coastline by Vancouver Aquarium Marine Mammal Rescue Centre staff, or were brought to the facility by members of the public, between June and September of 2007 (n=145) and 2008 (n=98). All pups were unweaned and estimated to be less than 10 days of age. Average body weight at admission was 7.8 ± 1.3 kg in 2007 (mean ± SEM; range 5.75-11.75 kg) and 7.7 ± 1.3 kg in 2008 (range 5.4-11.4 kg).

Animals were kept at the Vancouver Aquarium's Marine Mammal Rescue Centre following the standard operating procedures of the facility. They were housed individually in plastic tubs (approximately 61 x 92 x 61 cm). Tubs were kept under large tents with walls that could be raised and lowered to moderate ambient temperature. Tubs were cleaned daily, and in 2008 raised plastic grates were installed 5 cm above the bottom of the tubs to provide a cleaner surface for the pups to rest on. Animals were allowed to swim 1-4 times per day for durations of 2-20 minutes, by filling tubs with fresh water and then draining them. This was not done if pups were injured or sick, had full lanugo, or were very thin (scored < 2 out of 5 for body condition). Lanugo is soft, silvery white fur, and although approximately 21% of pups are born with it, it is typically shed in utero (Cottrell *et al.* 2002). When animals were healthy (normal blood values, not receiving medical treatments) and weaned, they were housed in fiberglass pre-release pools (approximately 22,775 L, 4.87 m x 1.21 m with a 2.34 m x 1.21 m haul-out) in groups of up to 8.

Pups admitted to the facility were randomly assigned to one of two diets and fed until weaning at roughly 20 - 30 days of age. In 2007, 73 pups were assigned to diet 1, and 72 to diet 2. In 2008, 46 pups received diet 1 while 47 were allocated to diet 2.

Diet 1 consisted of 1.0 kg commercial pinniped milk-replacer (Zoologic® 30/55 Milk Matrix, PetAg, USA, Appendix A), plus 2.5 L water, 800 ml salmon oil and 4 vitamin tablets (Mazuri Maz® Vita-Zu mammal tablets, PMI Nutrition, USA) per batch. Diet 2 was fish-based, consisting of 1.5 kg ground herring (fins and tails removed), 2 L water, 800 ml salmon oil and 4 vitamins tablets per batch. Formulas were made daily, refrigerated, and used for up to 24 h after herring was thawed or after milk-replacer was made. Representative samples of formulas were taken 2 times per week, and these weekly samples were pooled from July to September to give one representative sample for nutrient composition analysis each year. The herring used in each year was also analyzed. Proximate analysis is shown in Table 2.1.

Upon arrival at the facility, pups were first rehydrated by gavage feeding with an electrolyte solution, and then were gradually introduced to full formula by mixing formula with the electrolyte solution in subsequent feeds. Pups had usually progressed to the full formula by their 10th feed after admittance in 2007 and by the 5th feed in 2008.

In 2007, most animals were fed formula 4 times per day at 0715 h, 1300 h, 1600 h and 1815 h with a water feed at 1000 h, although some of the pups were fed formula at all five feeds. Target feed amount was 8% of their body weight per day (80 g formula/kg body weight), except on days when formula was reduced or stopped because of vomiting.

The feeding strategy was similar in 2008, except feeding times were at approximately 0700 h, 1030 h, 1400 h, 1700 h and 1930 h. As well, all 5 daily gavage feeds consisted of formula. In 2008, target feed amount was 11% of pups' body weight (110 g/kg body weight) per day.

Pups were weaned onto whole herring when they were estimated to be between 20 and 30 days old, had shed their lanugo, and/or their teeth had erupted. Weaning occurred by transitioning animals from formula to whole herring by gradually substituting herring for formula over approximately 5 days. An animal's weaning day was considered to be the first day on which every feed consisted only of whole herring. If an animal was not weighed on its weaning day, its last weight before weaning was taken as its weaning weight.

Because exact body weight was not known for each pup on each day, the amount to be fed each day was estimated by animal care staff. To confirm the actual amounts fed as a proportion of body weight, detailed records of every feeding were assembled for 48 animals (24 in 2007 and 24 in 2008) that survived until release. Mean food amounts per body weight for both formulas, and for the whole-herring diet fed after weaning, were calculated from the average of the actual food amounts provided to these animals. The analysis confirmed that formula was fed at 8 ± 0.2 % (mean ± SEM) and 11 ± 0.3 % of body weight per day in 2007 and 2008 respectively, and that whole herring was fed to weaned pups at approximately 13 ± 0.3 % of body weight per day in 2007 (130 g herring/kg body weight) and at approximately 11 ± 0.2 % per day in 2008 (110 g/kg body weight).

All animals were weighed at admittance (day 0) and then twice per week until release. Weights were measured on a digital scale (model 205, Cardinal Scale Manufacturing, Webb City, Missouri) accurate to the nearest 10 g, at approximately 0900 h on the designated days. As most pups had wet fur at the time of weighing, those animals that were dry were wetted for consistency. Additional weights were taken as needed for animals that were ill or had lost weight since last being weighed.

Statistical analysis

Non-parametric tests were used for statistical analysis because the data did not conform to the assumptions of parametric tests. Animals were not included in this analysis if they died before being fed full formula for at least 1 day or if they were severely injured (injured in such a way that may have impaired normal growth).

Mortality data (the number of pups on the two diets that died before weaning versus the number that survived to release) were analyzed by a chi-squared test (2007 n=113, 2008 n=69). Animals that died between weaning and release were excluded from this analysis because any influence of the pre-weaning diet on survival between weaning and release was uncertain.

The Mann-Whitney *U* Test was used to compare the average daily weight change of pups on the two diets for those animals that survived until weaning (2007, n=44, 2008 n=59). Two animals were excluded from the analysis because they were switched from one formula to the other.

To compare weight gains shortly before and after weaning, a subset of 60 animals (2007, n=24, 2008, n=36) with complete weight data for the 15 days before and the 15 days after weaning was assembled. Rate of weight change during these two periods was compared by the Wilcoxon Matched-Pairs Signed-Ranks Test. Weight change while on formula was calculated as body weight at weaning minus body weight at 15 days before weaning. Weight change during the 15 days after weaning was calculated as the difference between an animal's weaning weight and its weight on the weighing day closest to 15 days later. Values were expressed as change in body weight per day. Actual daily intake was calculated from feeding records for the same animals over the same two 15-day periods before and after weaning. Days when pups were transitioned to full formula, and from formula to whole herring, and days on which an animal vomited, were excluded.

2.3. Results

Mortality rates

Pups had relatively high mortality in 2007 (69%), but with an increase in feeding level and some improvements to hygiene, mortality rates improved dramatically in 2008 (20%). In 2007, the mortality rate of pups fed milk-replacer (60%) was lower than that of those fed fish formula (79%, $X^2 = 3.89$, $p = 0.05$, Table 2.2). In 2008, far fewer pups died (17% on milk-replacer, 24% on fish formula, $X^2 = 0.12$, n.s.). The combined data for both years showed a trend for lower mortality on milk-replacer ($X^2 = 3.17$, $p < 0.10$, Table 2.2).

Weight changes

On average, the unweaned pups gained weight on milk-replacer but lost weight on fish formula in both 2007 and 2008 ($p < 0.001$ in both years, Table 2.3). The informal comparison of the two years showed much greater gains in 2008 than 2007 for pups fed milk-replacer (Table 2.3). Pups fed fish formula lost slightly less weight in 2008 than in 2007 (Table 2.3).

In 2007, the pups with complete records for 15 days before and after weaning showed little weight gain on either diet in the 15 days before weaning, but pups on both diets gained weight at a faster rate in the 15 days after weaning ($p < 0.01$, Table 2.4). Food intake records showed that pups on milk-replacer experienced a 12 % increase in daily calorie intake at weaning (from 2264 to 2559 calories per day) while pups fed fish formula increased by 20 % (from 1878 to 2341, Table 2.4). In 2008, pups fed milk-replacer showed a small and non-significant ($p>0.05$) reduction in average daily weight-gain from the 15-day period before weaning to the 15-day period after, even though their daily calorie intake declined by 36 % (from 2879 to 1853 calories, Table 2.4). The average daily gain of those started on fish formula increased significantly after weaning ($p < 0.01$, Table 2.4) even though their daily calorie intake declined by 25 % when switched to whole herring (from 2318 to 1748, Table 2.4).

2.4. Discussion

The data on both weight gain and mortality indicate that the milk-replacer formula was more successful than the fish-based formula. Animals fed milk-replacer had modest weight gains (43 g/d in 2007, and 123 g/d in 2008), whereas pups fed the fish formula actually lost weight from admittance to weaning in both years. Additionally, in 2007 pups fed milk-replacer had lower mortality (60%) then those fed fish formula (79%) although this difference largely disappeared in 2008 when mortality rate was low on both diets.

Harbour seal milk consists of approximately 40% fat at parturition and increases to approximately 50% by day 7 where it remains until pups are weaned. Protein content of milk is slightly elevated at the time of parturition but is approximately 9% over the course of lactation (Lang *et al.* 2005). Neither of the diets in this study matched the fat and protein content of seal milk, but the milk-replacer, with 30% fat and 7.7% protein, was a better approximation than the fish formula with only 21.4% fat and 6.1% protein. The higher fat content of the milk-replacer was likely responsible for its higher energy concentration compared to fish formula (3.1 cal/g vs. 2.3 cal/g). This may have contributed to the increased weight gains of animals fed milk-replacer.

Both weight gain and survival showed a striking improvement from 2007 to 2008. On average, animals in 2008 gained substantially more on milk-replacer, and lost slightly less on fish formula, than in 2007. The difference is probably due to the greater calorie intake in 2008 caused by feeding formula at 11% of body weight rather than 8%.

Mortality rate was also much reduced in 2008 (20%) compared to 2007 (69%). Among mother-reared pups, estimates of mortality from parturition until weaning range from 12 to 31% (Steiger *et al.* 1989). Higher mortality rates are expected for pups admitted to rehabilitation facilities because the animals are often presented in poor physical condition. Mortality of rehabilitated pups varies widely between facilities and between years at the same facilities (Dubois 2003). For example, at one marine mammal facility, the percentage of pups successfully released each year ranged from 15% to 63% over a 5-year period (Lander *et al.* 2002). However, given that the pups consumed more food and gained more weight in 2008, it seems likely that the improved survival was due at least partly to improved nutrition.

Energy content of seal milk at day 7 of lactation is 21.9 MJ/kg or about 5.23 cal/g (Lang *et al.* 2005). Thus an intake of 1.2 -1.5 kg of seal milk per day, corresponding to milk intakes in the wild (W. D. Bowen personal communication, June 9, 2008), would result in an intake of approximately 6000 - 8000 calories/day. Animals in this study received far fewer calories in both years (Table 2.4), especially in 2007 when pups were fed at approximately 8% of body weight. The increased intake in 2008 appears to have resulted in improved pup performance from the previous year, but the rate of weight gain was still considerably less than that of mother-reared animals. Even greater intakes and more energy-dense diets may help. However, animals may be limited in their ability to digest fats and other nutrients that do not correspond to the chemical structure of their natural diet. Feeding at an increased frequency may result in too much handling and stress to the animals, and there is likely a physical limit to the volume that can be fed at any single feeding. Consequently, a delicate balance may need to be found between under-feeding versus feeding too much of a diet that is difficult for harbour pups to digest.

The change in weight gain at weaning suggests that another factor may be involved. After weaning, animals in both years typically gained 100 - 170 g/day on whole herring, even though herring is much lower in fat (12%) and thus lower in calories (1.75 cal/g, 5.6 cal/g DM) than the artificial diets. The large improvement in average daily gain after weaning in 2007 may be explained in part by the higher rate of intake after weaning, but the dramatic increase in weight gain was accompanied by only a modest increase in calories (Table 2.4). In 2008, when formula was fed at a higher level, the pups gained as well or better after weaning despite a large decrease in calorie intake. Most interestingly, the pups did notably better when fed whole fish by mouth than when fed the fish-based formula (ground herring plus other nutrients) by gavage.

A possible explanation is that gavage feeding itself may be part of the problem. As early physiological research showed, the anticipation of eating triggers the digestive process of salivation and secretion of stomach acids, but a stress-producing event can bring these processes to an abrupt halt (Canon 1915). Gavage is a stressful event involving restraint and forced insertion of the tube into the esophagus and stomach. This process took place 5 times per day and could indeed have inhibited digestion. In mice, force-feeding has been demonstrated to exacerbate illness. For example, mice infected with *Listeria monocytogenes* had shortened survival times and 50% increased mortality when force-fed rather than fed *ad libitum* (Murray and Murray 1979). A more natural feeding system might contribute to greater assimilation of the formulas and increased weight gain.

Providing hand-reared harbour seal pups with a diet that maximizes weight gains is important for their success, as they must achieve sufficient body fat and weight if they are to survive once released. Harding *et al*. (2005) estimated that harbour seal pups must weigh 26 kg by autumn if they are to survive their first winter, and that heavier pups generally had a greater chance of surviving their first year. There is also evidence of a positive correlation between weaning weight and survival in the first year for southern elephant seals (*Mirounga leonina*; McMahon *et al.* 2000), northern fur seals (*Calorhinus ursinus*; Baker and Fowler 1992) and grey seals (*Halichoerus grypus*; Hall *et al.* 2001). Although there have been no studies specifically on rehabilitated seals, it seems likely that bigger and fatter pups may have an enhanced chance of survival as their greater energy stores will better protect them from cold ocean temperatures and sustain them longer while they learn to forage.

2.5. Conclusions

Based on the results of this study we conclude: (1) although neither diet achieved the weight gains recorded in mother-raised pups (400 – 800 g/d), the artificial milk formula was clearly more successful in terms of higher weight gains and improved survival; (2) the higher feeding rate used in 2008 (11% of body weight per day) supported greater gains and improved survival rate; (3) more work is needed on both diet composition and feeding method to achieve more natural weight gains.

Table 2.1. Proximate analysis of hand-rearing formulas and of herring fed to harbour seal pups at Vancouver Aquarium Marine Mammal Rescue Centre in 2007 and 2008.

Component	Milk-replacer		Fish formula		Solid herring	
	2007	2008	2007	2008	2007	2008
Moisture (%)	60.1	58.7	69.0	69.7	69.7	67.8
Dry Matter (DM,%)	40.0	41.3	31.0	30.3	30.3	32.2
Fat (%)	30.3	30.0	21.6	21.1	11.5	12.6
Protein (%)	7.8	7.6	6.3	5.9	16.4	17.0
Ash (%)	1.5	1.6	0.5	0.5	2.4	2.6
Carbohydrate (%)	0.4	2.1	2.7	2.8	<1.0	<1.0
Energy (kcal/g, wet)	3.1	3.1	2.3	2.3	1.7	1.8
Energy (kcal/g, DM)	7.6	7.5	7.4	7.4	5.6	5.6

Table 2.2. Number of harbour seal pups that survived until or died before weaning when fed milk-replacer or fish formula in 2007, 2008, and both years combined.

Outcome	Diet		P*
	Milk-replacer	Fish formula	
2007			
Survived	23	12	≈ 0.05
Died	34	44	
2008			
Survived	29	26	n.s.
Died	6	8	
Total			
Survived	52	38	< 0.10
Died	40	52	

*X^2 Test (Siegel, 1956)

Table 2.3. Mean (± SEM) daily weight change (g/day) for harbour seal pups fed milk-replacer or fish formula until weaning in 2007 and 2008.

Year	Diet		P*
	Milk-replacer	Fish Formula	
2007	43 ± 12 (n=27)	-13 ± 6 (n=17)	< 0.001
2008	123 ± 12 (n=29)	-6 ± 8 (n=30)	< 0.001

* Mann-Whitney *U* Test (Siegel, 1956)

Table 2.4. Weight change (g/day) and estimated daily intake (calories/day) in the 15-day period immediately before and immediately after weaning, for harbour seal pups fed milk-replacer or fish formula in 2007 and 2008. Values are mean ± SEM.

	Number of pups	Mean daily weight change (g/d)			Estimated daily intake (calories/day)	
		Before weaning	After weaning	P*	Before weaning	After weaning
2007						
Milk-replacer	16	25 ± 14	157 ± 14	<0.01	2264 ± 95	2559 ± 274
Fish formula	8	2 ± 9	170 ± 34	<0.01	1878 ± 46	2341 ± 274
2008						
Milk-replacer	18	102 ± 13	96 ± 10	n.s.	2879 ± 62	1853 ± 52
Fish formula	18	10 ± 12	113 ± 9	<0.01	2318 ± 52	1748 ± 67

*Wilcoxon Matched-Pairs Signed-Ranks Test (Siegel, 1956)

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Chapter 3: The provision of supplementary heat for hand-raised harbour seal pups (*Phoca vitulina*)[2]

3.1. Introduction

In British Columbia many harbour seal pups (*Phoca vitulina*) are stranded every year and several hundred are admitted to wildlife centres for rehabilitation. These animals are usually unweaned, and are often ill, injured or in poor body condition. In captive care they can have low weight gains (Duerr 2002; Wilson *et al.* 1999) and high mortality rates in certain years (Lander *et al.* 2002). Weight of the pups at admittance to rehabilitation facilities is often lower than published mean birth weights (Larmour 1989), likely because of separation from their mothers. Pups in poor body condition with a thin blubber layer may have difficulty maintaining core body temperature (Markussen *et al.* 1992, Bowen et al. 1994).

At birth harbour seal pups have approximately 11% body fat with a blubber layer that is approximately 1.4 cm thick (Bowen *et al.* 1991). This fat is a source of stored energy and provides insulation (Oftedal *et al.* 1991). Having an adequate blubber layer is particularly important as harbour seal pups are precocial and often follow their mothers into cold ocean water hours after birth (Lawson and Renouf 1985). Pups in poor body condition have a relatively higher surface-to-volume ratio compared to animals with adequate fat stores and may have to expend more energy to maintain normal body temperature (Bowen *et al.* 1994).

Supplementary heat is used in rearing young mammals of many species and has been shown to help promote growth and survival (Curtis 1983). Additionally, many mammals will seek heat when ill (Akins *et al.* 1991; Kleitman and Satinoff 1981). Harbour seal pups presented

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to rehabilitation facilities in poor body condition may also benefit from the provision of a supplementary heat source. Behavioural observations conducted at the Vancouver Aquarium Marine Mammal Rescue Centre showed that seal pups at the facility frequently shivered or adopted body positions likely to conserve heat (hunched posture, flippers tightly tucked under or against body).

The objectives of this study were to determine: (1) whether animals provided with supplementary heat would use it in an adaptive manner, and (2) whether animals provided with supplementary heat would have greater weight gains and higher survival rates until weaning when compared to animals without heat.

3.2. Materials and methods

Animals

A total of 98 stranded harbour seal pups were recovered along the British Columbian coastline by staff of the Vancouver Aquarium, or were brought to the facility by members of the public, between June and September of 2008. All pups were unweaned and estimated to be less than 10 days of age. Body weight at admission averaged 7.7 ± 1.3 kg (mean ± SEM, range 5.4-11.4 kg).

Housing and handling

Animals were kept at the Vancouver Aquarium's Marine Mammal Rescue Centre following standard procedures of the facility. They were singly housed in plastic tubs (approximately 61 x 92 x 61 cm) with plastic floor grates raised 5 cm above the floor of the tub. Tubs were kept under large tents with walls that could be raised and lowered to moderate ambient temperature. Enclosures were cleaned daily. Once the pups were weaned onto a diet of whole fish and were healthy (normal blood values, not receiving medical treatment) they were moved to fiberglass pre-release pools (approximately 23,000 L with a haul-out area) in groups of up to 8.

Eight of the tubs were equipped with supplementary heat provided by Canarm® infra-red brooder heat lamps (model HLC, Brockville, Ontario) with Philips infra-red 175-watt bulbs. Lamps were hung from overhead cables. They were positioned 46 cm above the plastic floor-grates so that pups could not touch them, and they could be raised clear of the tubs when necessary. Lamps were positioned centrally at one end of each tub (either west or east) so that a thermal gradient was established between the heated half and the unheated half of the tub.

Radiant temperature (the temperature likely to be perceived by the animal) was determined using a black globe thermometer (model 210-4417, Novalynx Corporation, Grass Valley, California) in each half of the enclosures for a series of ambient environmental temperatures. On average, radiant temperature was 9˚ C higher than ambient at pup height directly under the lamp and approximately 6.7˚ C higher in the remaining area of the heated half of the enclosure. Radiant temperature was approximately 2.2˚ C higher than ambient in the unheated half of the enclosure. Thus, mean radiant temperature was approximately 6˚ C higher on average in the heated half than in the unheated half.

All animals were weighed (in tared plastic totes) at admittance (day 0) and then twice per week until release. Weights were measured on a digital scale (model 205, Cardinal Scale Manufacturing, Webb City, Missouri) accurate to the nearest 10 g, between the first and second feeds. Most pups had wet fur at the time of weighing. If animals were not already wet, the fur was wetted for consistency. Additional weights were taken as needed for animals that showed signs of illness or had lost weight since last being weighed.

As part of another study (Chapter 2), pups were randomly assigned at admittance to one of two diets, either an artificial milk replacer (30% fat, 7.7% protein, 3.1 cal/g) or a fish-based formula (21.4% fat, 6.1% protein, 2.3 cal/g) as described by MacRae *et al.* (2010). Pups were fed formula via gavage at approximately 0700 h, 1030 h, 1400 h, 1700 h and 1930 h. Upon arrival at the facility, they were first rehydrated by gavage feeding with an electrolyte solution, and then were gradually switched to full-strength formula by mixing formula with the electrolyte solution in subsequent feeds. Most animals were on full formula by their fifth feed after admittance. Pups were fed approximately 11% of body weight (110 g/kg body weight) per day. Pups were weaned onto whole herring when they were estimated to be between 20 and 30 days old, had shed their lanugo, and/or their teeth had erupted.

Because heat was expected to be most beneficial for underweight animals, the experiment was limited to pups with admission weights of < 9 kg, and body condition scores of 1 or 2 on a scale of 1 to 5 where 1 is emaciated. All pups were judged to be < 10 days old. Any pups with serious injuries were omitted. Sixty-seven animals met these criteria. When an animal meeting these criteria was admitted to the facility, it was placed in a heated tub if one was available. Because only 8 tubs had heat, the next 1-2 similar pups were typically assigned to unheated tubs. In total 25 pups were kept in heated tubs and 42 in unheated. Heat was provided for 21 days. Ambient temperature in the facility was recorded at the start of every observation session using a digital maximum/minimum thermometer. Heat lamps were turned off at the end of daily observation sessions or any time that ambient temperature reached 25˚C. Pups were carefully monitored for signs of heat stress during the day. Lamps were also turned off and raised during cleaning and when pups had swimming sessions. Every night lamps were lowered and turned on via a timer at 21:00 so pups had heat overnight.

During the 21-day period that animals received supplementary heat, observations of animal position and orientation in relation to the heat source were recorded daily from 07:00

until 11:00 at 15-min intervals. A pup was scored as being in the heated half of the tub if it had more than 50% of its body in that half.

After the third feed (14:00) each day, pups were usually given a swim for durations of 2-20 min, by filling tubs with fresh water and then draining them. After tubs had been drained, heat lamps were turned on for 12 min. Observations of pup position (heated or unheated side) were recorded every minute for 10 min in the same manner described above, starting 2 min after the heat lamps were turned on. Lights were turned off and raised after observations were completed. Pups were not allowed to swim if injured or sick, or if they had full lanugo.

Statistical analysis

Animals were not included in this analysis if they died within the first 2 days of being admitted. Of the 25 animals provided with heat, one did not have a complete set of behavioural observations so was removed from the behavioural portion of the analysis. Data from the 24 animals observed with heat from day 0 (admittance) to day 21 were used to determine if animals would seek heat at cooler ambient temperatures. Ambient temperature was first categorized into the following temperature ranges: (1) < 16 ° C; (2) 16 – 18.9 ° C; (3) 19 – 21.9 ° C; and (4) ≥ 22° C. The proportion of observations that seals were in the heated half of the tub was calculated for each seal at each temperature range over the 21 days. Five animals had fewer than 10 body position observations for one temperature range; in these cases those observations were excluded but the remaining three temperature ranges for those animals were included. The effect of temperature category (categorical, df=3) on the percentage of observations in which an animal was under the heat source was then analyzed using a mixed model (SAS v9.1).

A one-tailed binomial test was used to test whether pups positioned themselves more on the heated half of their tub during observations taken after swimming in cold water.

X^2 analysis was used to compare survival of animals with or without supplementary heat. It was based on the 67 animals (heat n=25, no heat n=42) that either survived until or died before day 21.

The Mann-Whitney *U* Test was used to compare the average daily weight change of pups with and without heat; a separate analysis was used for each diet (milk-replacer and fish formula). The analysis was based on seals that survived until weaning. These included 9 pups fed milk-replacer with heat and 15 without heat, and 10 pups fed fish formula with heat and 14 without heat.

3.3. Results

Heat-seeking behaviour was observed in pups at low ambient temperatures and declined as temperature increased. Animals spent 61 ± 4 % (mean ± SEM) of observations on the heated side of the enclosure when ambient temperature was < 16 ° C. This value declined steadily with increasing ambient temperature, to only 36 ± 4 % when temperature reached ≥ 22° C (Figure 3.1). Statistical analysis showed differences between the four temperature categories in the percentage of observations in which animals were on the heated side ($p < 0.001$).

One to sixteen observation sessions were conducted immediately after swim sessions for 19 of the animals provided with heat. The pups spent a mean (± SEM) of 67 ± 6 percent of observations on the heated side of the enclosure, with 14 of the 19 seals spending more than 50% of observations after swims on the heated side ($p = 0.032$, binomial test, one-tailed). The effect was particularly clear for the 6 seals that had the most experience of swimming sessions: all 6 pups that had more than 10 swims were observed more on the heated side ($p = 0.016$, binomial test, one-tailed).

Mortality rate was low (8%) for larger pups (>7 kg) and identical for both heat and no-heat treatments (Table 3.1). For smaller pups, more died in the no-heat treatment (8/18) than in the heat treatment (3/13), but the difference was not significant by the X^2 test (X^2 =0.72, p ≈ 0.2, one-tailed; Table 3.1).

The pups fed milk-replacer had extremely variable changes in body weight, but most gained weight. There was no difference between those provided with heat and those without (Table 3.2). Pups fed fish formula had little or no weight gain. Gains averaged (13 ± 7 g/day) for pups provided with heat but pups lost weight on average (-3 ± 8 g/d) without heat (p ≈ 0.10; Table 3.2).

3.4. Discussion

This study showed that if heat is provided at one end of a small enclosure, pups will position themselves in a seemingly adaptive manner, clearly using heat when ambient temperature was below 16 ° C and clearly avoiding it when ambient temperature was 22 ° C or higher. The pups also selected the heated area just after being cooled by swimming in cold water. As water has twenty times the thermal conductivity of air it is challenging for pups to conserve heat while in water (Harding *et al.* 2005). Small animals without a store of insulative fat may have difficulty thermoregulating and may be in a negative energy balance when exposed to cold temperatures. Moving close to a heat source when ambient temperatures are low or after being chilled from swimming likely represents an attempt at energy conservation.

Data on survival and weight gain provided no definitive differences between treatments, but were consistent with the hypothesis that heat may benefit smaller malnourished animals. First, there was a trend for better survival among small pups (≤ 7 kg) if they had heat. Smaller animals may benefit more from a heat source as they must expend more energy for thermogenesis (Harding *et. al.* 2005). An animal's thermo-neutral zone (the range of ambient temperatures at which no additional metabolic energy is required to maintain body temperature) is a function of body size, level of insulation, and the ratio of body surface area to volume (Bartholomew 1977). Outside its thermo-neutral zone an animal must increase its metabolic rate to regulate its temperature (Worthy 2001). Small animals with a greater ratio of surface area to volume experience greater rates of heat loss, and a seal with inadequate blubber stores may be more susceptible to thermal challenges.

Secondly, there was a trend ($p \approx 0.10$) for animals fed the less nutritious fish formula to gain more weight when provided with heat. Immature animals require additional energy above their maintenance requirements for growth (Worthy 2001, Lavigne *et al.* 1986). When compared to the 400-800 g/day gains of mother-raised animals (Cottrell *et al.* 2002; Bowen *et al.* 1994), neither artificial diet seems to provide adequate energy to sustain pups' metabolic maintenance and growth requirements. In particular, the fish formula is a poor approximation of the fat-rich (40%), energy-dense harbour seal milk (Lang *et al*. 2005). Animals fed fish formula had a lower nutritional plane, and thus may have benefited more from the provision of heat as indicated by a slightly greater weight gain ($p \approx 0.10$).

Although only moderate ambient temperatures were recorded during the study period, the results allow cautious extrapolation. Presumably the supplementary heat would be used even more in colder conditions, and in fact seals were consistently seen directly under the heat lamps during occasional observations during cool night-time hours. The results also suggest that the practice of turning lamps off on warm days (above 25° C) was warranted.

3.5. Conclusions

We conclude that harbour seal pups reared for release prefer, and may benefit from, access to a supplementary heat source, especially in the case of animals in poor body condition fed low calorie diets and maintained at cool ambient temperatures.

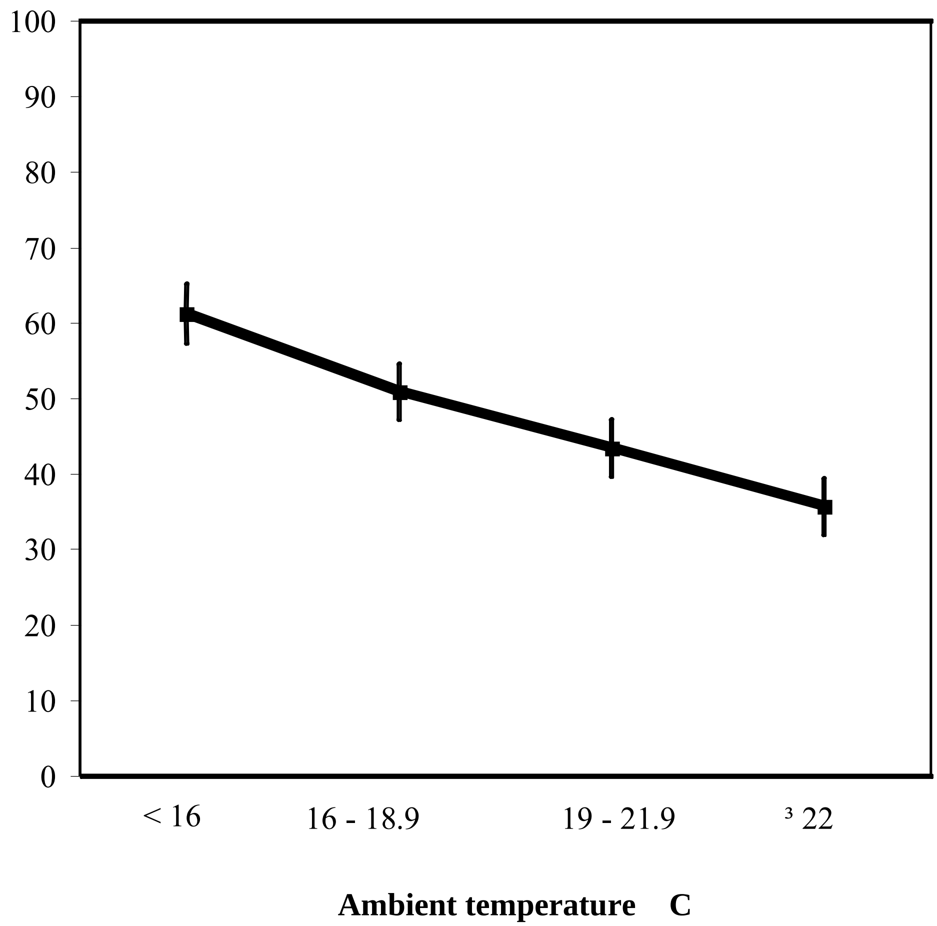

Figure 3.1. Percent of observations (mean ± SEM) measured by 15-min scan sampling that 24 harbour seal pups were observed on the heated side of their enclosures at four categories of ambient temperature.

Table 3.1. Number of harbour seal pups weighing ≤ 7 kg or > 7 kg that lived or died before day 21 with or without a supplementary heat source[3].

	Heat			No heat		
Size Category	Lived	Died	% Died	Lived	Died	% Died
Pups ≤ 7 kg*	10	3	23 %	10	8	44 %
Pups > 7 kg	11	1	8 %	22	2	8 %

*X^2 =0.72, p ≈ 0.2, one-tailed (Siegel, 1956)

[3] Pups with heat n=25 of which 13 ≤ 7 kg and 12 > 7 kg, pups without heat n=42 of which 18 ≤ 7 kg and 24 > 7 kg.

Table 3.2. Mean (± SEM) daily weight change (g/d) for harbour seal pups with and without supplementary heat fed milk-replacer or fish formula until weaning (n=24 milk replacer, n=24 fish formula).

	Treatment		
Diet	Heat	No heat	P*
Milk-replacer	98 ± 21 n=9	142 ± 14 n=15	n.s.
Fish formula	13 ± 7 n=10	-3 ± 8 n=14	≈0.10

* Mann-Whitney *U* Test (Siegel, 1956). One-tailed test was used because the hypothesis predicted greater weight-gain with heat than without.

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Chapter 4: General discussion and conclusions

4.1. General discussion

For the successful rehabilitation of harbour seal pups, care in captivity must result in satisfactory health and weight gain. For unweaned harbour seal pups, this requires hand-rearing formulas that promote rapid fat deposition so that the animals are equipped with the blubber that will provide both thermal insulation and a source of stored energy once released.

Meeting the energy requirements of growing seal pups is challenging and requires attention to both the amount consumed and the energy density and composition of the diets being fed. Both the milk replacer and the fish formula used in this study were much lower in caloric content than seal milk, making it difficult to meet pups' nutritional needs. Increasing the amount fed from 8% of body weight daily in 2007 to 11% in 2008 improved weight gains and mortality rates for animals on both diets. However, increasing intake via either meal size or feeding frequency is limited by the stomach size and digestive capacity of pups. Pups fed milk-replacer did have higher weight gains and lower mortality than animals fed fish formula, likely due at least in part to its higher caloric value. Yet, even when being fed the more energy-dense milk-replacer at the higher intake level (11%), pups had rates of weight gain much lower than mother-raised animals.

It is possible that in addition to being low in energy, the composition of the artificial diets may have been a factor in pups' ability to digest, and thus utilize, the nutrients. Young mammals have digestive systems adapted to a diet of their mother's milk. For harbour seal pups this means the ability to assimilate large amounts of milk fat. Eighty-five to 95% of the calories consumed by seal and sea-lion neonates come from milk lipids (Iverson *et al.* 1992). These milk lipids are characterized by long-chain polyunsaturated fatty acids directly derived from the marine diet (Iverson *et al.* 1992). The ability of pups to rapidly deposit blubber suggests they are able to efficiently absorb these lipids (Bowen 1985).

The digestion of fats depends on lipases, enzymes that act at specific positions on the glycerol backbone of the lipid substrate and on specific fatty acids (Iverson *et al.* 1992). The specificity of fat digestion is demonstrated by work done with piglets; these animals are able to digest their mothers' milk which is rich in fat, but when fed artificial diets rich in fat of a different chemical nature they often develop diarrhea, depressed growth and may even die (Manners *et al.* 1976). The composition of the fats in the artificial diets fed to rehabilitated pups may be different enough from seal milk lipids that that they cannot be absorbed as efficiently, and thus utilized. Instances of diarrhea and "greasy feces" (containing undigested salmon oil), were frequently noted for pups on both diets during this study, perhaps indicating poor absorption. It is also possible that pups on milk-replacer had better weight gains not only because of the higher caloric content, but because they were better able to digest the milk-replacer than the fish-based formula.

The assimilation of the diets may also have been affected by the gavage feeding method. Fear-producing situations can inhibit or terminate the digestive processes through activation of the sympathetic nervous system (Fraser 2008). The stress presumably associated with restraint and force-feeding of pups may inhibit their digestion and subsequent assimilation of their food. Supporting this conjecture is the interesting result discussed in Chapter 2, in which animals gained weight relatively better when fed the lower-calorie herring diet by mouth, than either of the higher-calorie formulas via gavage.

In addition to being nutritionally challenged, some of the animals admitted to rehabilitation may have difficulty maintaining body temperature. As noted in Chapter 3, small pups, without an adequate layer of fat, may have difficulty maintaining thermal stability and in some cases may be unable to keep heat production in balance with heat losses (Markussen *et al.* 1992; Bowen *et al.* 1994).

The process of digestion increases metabolism and generates additional heat, often called the heat increment of feeding (Worthy 2001). The rate and duration of the increased metabolism depends on the substances consumed. For example, metabolic rate can be increased 4 to 30 % for 2 to 5 hours by carbohydrates, 30 to 70 % for up to 12 hours by proteins, and 4 to 15 % for 7 to 9 hours by lipids (Hoch 1971). Compared to mother-reared pups consuming fat-rich milk (Iverson *et al.* 1992), the orphaned pups, being fed the lower-fat artificial diets (30% fat in milk-replacer, 21% fat in fish formula) would not be expected to produce the same heat from digestion as animals fed their mother's milk. Less heat produced from digestion, combined with increased heat loss due to poor body condition, presents an energy challenge for these animals.

Supplementary heat has been shown to promote growth and survival in young mammals of many domestic species (Curtis 1983) and to prolong the life of both rats (Kleiber 1961) and piglets (Morrill 1952) during starvation. The provision of heat pads has been suggested to reduce calorie consumption for seal pups that are severely emaciated or still have lanugo (Townsend and Gage 2001). We therefore hypothesized that a source of radiant energy in the form of heat lamps would help compensate for the energy challenge faced by pups in poor body condition, and thus help reduce their energy expenditure. For the purpose of this study, heat lamps were more practical than heat pads as they could be used without coming into physical contact with the pups or with water.

Although there was not a significant difference in mortality rate or weight gains between animals with and without heat, there was a trend for lower mortality for lighter pups (≤ 7kg). These animals likely experience a greater rate of heat loss due to their larger surface to volume ratio (Bowen *et al.* 1994) and lower percentage of body fat, so may have benefited more from the heat lamps. As well, the animals fed fish formula showed a trend for less weight loss with a heat lamp than without. This is consistent with the hypothesis that the heat lamp aided in energy conservation.

4.2. Recommendations and future directions

Harbour seal pups are being successfully raised and released in rehabilitation facilities but there is still a need for refinement of certain aspects of their care. Based on the results from this study, I recommend that until a more calorie-dense, easily digestible diet is developed, milk-replacer should be fed instead of fish formula to unweaned harbour seal pups, and that intake levels be increased as much as is feasible without causing illness.

My results also suggest that the use of heat lamps, particularly for small animals, is a good management practice since seal pups show a strong preference for this external source of heat at temperatures <16 °C. The provision of heat may be particularly advantageous for pups that that still have their fetal pelage or lanugo. Lanugo is soft, silvery white fur, and although approximately 21% of pups are born with it, it is typically shed in utero (Cottrell *et al.* 2002). Lanugo animals are considered to be premature, as they are often born early in the pupping season (when temperatures may be colder), and have lower than average birth weights and fat stores (Bowen *et al.* 1994). Additionally the lanugo coat only holds its insulative properties when dry (Oftedal 1991) meaning that these pups in particular may face thermal challenges.

Future research could explore alternative feeding methods. A striking feature of captive pups, noted throughout my two years of work, was persistent but highly variable non-nutritive sucking directed to pen walls or their own bodies. In other species, non-nutritive sucking or feeding actions may simply indicate hunger (Jensen *et al.* 1998) or they may be learned behaviour that helps trigger digestive processes in animals fed in unnatural ways (Wiepkema *et al.* 1987). Natural sucking behaviour has been shown in ruminants to increase the secretion of digestive hormones (de Passillé *et al.* 1993). As a possible explanation for the better weight gains seen in animals once weaned onto the lower-calorie herring diet, stress associated with gavage feeding may be interfering with digestion. If this proves to be true, then development of an alternative feeding system based on sucking instead of via gavage, would have the potential to improve digestion, reduce stress from forced-feeding and thus improve pup growth and welfare.

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Appendices

Appendix A: Milk matrix 30/55 typical nutritional analysis

Composition per 100 grams powder			
Energy kcal	595.0	Fiber g	0.0
Protein g	31.3	Moisture g	3.6
Fat g	55.8	Ash g	7.0
Carbohydrate g	3.1		
Minerals per 100 grams powder			
Calcium mg	1090.0	Iron mg	11.0
Phosphorus mg	753.0	Copper mg	1.0
Potassium mg	805.0	Zinc mg	6.0
Sodium mg	575.0	Manganese mg	1.4
Magnesium mg	88.0	Total Chloride mg	741.0
Vitamins per 100 grams powder			
Vitamin A I.U.	3656.0	Pantothenic Acid mg	7.0
Vitamin D3 I.U.	1023.0	Vitamin B6 mg	0.4
Vitamin E I.U.	34.4	Choline mg	498.0
Thiamin mg	0.4	Folic Acid mg	0.3
Riboflavin mg	0.9	Vitamin B12 mcg	6.0
Niacin mg	7.6	Biotin mcg	31.1
Amino acids per 100 grams powder			
Lysine g	2.15	Valine g	1.85
Arginine g	0.99	Histidine g	0.86
Methionine g	1.08	Alanine g	0.60
Cystine g	0.11	Aspartic Acid g	2.39
Tryptophane g	0.38	Glutamic Acid g	6.01
Threonine g	1.32	Glycine g	0.58
Isoleucine g	1.47	Proline g	3.07
Leucine g	2.41	Serine g	1.65
Phenylalanine g	1.55	Tryosine g	1.39
Fatty acids per 100 grams powder			
8:0 Caprylic Acid g	0.02	16:1 Palmitoleic Acid g	1.57
10:0 Capric Acid g	0.06	18:0 Stearic Acid g	6.94
12:0 Lauric Acid g	0.10	18:1 Oleic Acid g	23.31
14:0 Myristic Acid g	0.78	18:2 Linoleic Acid g	4.91
16:0 Palmitic Acid g	12.71	18:3 Linoleic Acid g	0.20
Guaranteed analysis			
Crude Protein %, min.	30.0	Moisture %, max.	5.0
Crude Fat %, min.	55.0	Ash %, max	8.0
Crude Fiber %, max.	0.25		

Ingredients

Animal fat (preserved with BHA and citric acid), casein, dicalcium phosphate, condensed whey, vegetable oil, calcium carbonate, lecithin, potassium chloride, choline chloride, magnesium sulfate, vitamin E supplement, vitamin A supplement, zinc methionine, ferrous sulfate, calcium pantothenate, vitamin B12 supplement, niacin supplement, manganese sulfate, copper sulfate, vitamin D3 supplement, riboflavin, thiamine mononitrate, pyridoxine hydrochloride, menadione sodium bisulfite complex, folic acid, calcium iodate, biotin, sodium selenite, mono and diglycerides.

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Appendix B: Photographs of the heat tent

Photograph 1. Heat tent

Photograph 2. Pup basking under heat lamp