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PREDICTORS OF WEIGHT GAIN IN THE REHABILITATION OF EASTERN NORTH ATLANTIC GREY

SEALS (HALICHOERUS GRYPUS).

by

SAMANTHA MCGIVERN

A thesis submitted to the University of Plymouth in partial fulfilment for the degree of

RESEARCH MASTERS IN BIOLOGICAL SCIENCES

School of Biological and Marine Sciences.

[In collaboration with Seal Rescue Ireland]

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**Author's Declaration:**

At no time during the registration for the degree of Research Masters in Biological Sciences has the author been registered for any other University award without prior agreement of the Doctoral College Quality Sub-Committee.

Work submitted for this research degree at the University of Plymouth has not formed part of any other degree either at the University of Plymouth or at another establishment.

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Samantha McGivern; Predictors of Weight Gain in the Rehabilitation of Eastern North Atlantic Grey Seals (*Halichoerus grypus*).

Abstract:

As large adult body size is subjected to strong selective pressures, other processes can be compromised before growth when there is resource limitation or stress during the growth phase. Stress has been linked with both weight loss and weight gain in a variety of taxa, and some stressors, such as physical disturbance, are known to affect the development of important physiological processes, e.g., diving physiology in marine mammals. With 40% of the world's grey seal (*Halichoerus grypus*) population on UK shores, grey seal pups are frequently admitted to rehabilitation centres within their first four weeks – a crucial growth phase – across the UK. Common rehabilitation practices (e.g., change in environment, social grouping, and disturbance) are known to cause stress to individuals in other contexts. Within grey seal rehabilitation, there are known factors outside the control of the centre that affect weight gain and survival, e.g., being underweight upon admittance. Yet, there are management practices, such as movement of individuals between pools (requires handling, movement, and regrouping) that may cause stress. The effects of these management practices on weight gain and mortality is unknown. By studying the behaviour of individuals, it can provide insights into how or why there are any changes in weight gain and mortality. This study investigates known factors outside the control of one rehabilitation centre over six pupping seasons, and factors inside the control of the centre on one case study year (detailed pool information available and behavioural data collected on <70 individuals across pools) on weight gain and mortality. No evidence was found that management practices (pool characteristics, pool movements) affected weight gain, which was explained by duration of rehabilitation, and admittance reasons: underweight and injured. However, a high number of pool moves is associated with mortality. Pools varied in movement, rest, and head elevated behaviour (may indicate disturbance), suggesting some characteristics such as the visual blockade, might modify stress exposure or energetic costs during growth. No clear pattern across pools with respect to weight and mortality emerged, suggesting that although there are indicators of disturbance it might not be affecting weigh and mortality. We recommend that the centre reviews the practice of pool moves and for all centres to include a visual blockade for seals in the design of pools to decrease the disturbance caused by high footfall areas.

# Contents:

## 1. Introduction:

Rehabilitation centres have become a key part of conserving wild populations (Aitken, 2004), mitigating the effects on populations from human disturbance and severe weather events, by rehabilitating and releasing individuals back into the wild (Cope et al., 2022). Grey seal (*Halichoerus grypus*) pups enter rehabilitation centres within their first four weeks – a crucial growth phase. Therefore, it is imperative that we understand the implications of management practices on growth. A recent review showed that there are many known factors that could affect weight gain and pup survival (e.g., intake weight, weight gain, admittance reasons, age, sex, and pupping seasons), providing management recommendations on protocols during the triage and treatment phase (Zatrak et al., 2022). However, there could be unknown management factors (e.g., routine movement between pools (part of standard management in captivity), other pool disturbances (e.g., staff within the pools and water drainage as part of cleaning (part of standard management in captivity), and other wildlife present), and duration of rehabilitation) that are not considered which could be impacting weight gain and survival of grey seal pups. Individuals are routinely moved between pools as part of standard management in captivity and it largely remains unknown what the impact of these moves have on individuals versus the variation in disturbance levels across pools within centres. This thesis will explore both the known and unknown factors to understand if there are further practices that should be considered when rehabilitating wild grey seal pups.

The introduction will cover relationships between weight and stress, hence how stress during management might affect the mortality and growth of seals. A further explanation on the relevant aspects of seal phenology and physiology, and an

introduction into rehabilitation centres (management practices, characteristics, and grey seal rehabilitation).

1.1: Weight and Stress:

Growth rate, according to life history theory, can be subjected to strong directional selection i.e., the reproductive and survival advantages associated with large body size including fecundity, mate selection, predation avoidance, and offspring survival (Dmitriew, 2011). However, optimal growth is seldom maximal, shaped by selection pressures to reduce the negative physiological impacts of rapid growth, such as reduced immune function due to resource allocation to growth, and reduced resistance to physiological stressors, for example drought due to decreased resistance to elevated sodium concentrations (Mangel and Stamps, 2001)

As growth is subjected to strong selective pressures that favour fast growth; other processes are often observed to be compromised before growth when there is resource limitation. The costs associated with rapid growth are not evident until specifically after reaching the reproductive phase (Hector and Nakagawa, 2012). For example, there is cumulative damage and development flaws evident in faster cellular senescence (Ben-Porath and Weinberg, 2004). In European shags (*Phalacrocorax aristotelis*), high levels of stress during early post-natal life has potentially been mechanistically linked to increased shorten telomeres in nestlings, reducing longevity (Herborn et al., 2014). Herborn et al., (2014) also found that it provided, at minimum, evidence of oxidative damage resulting from oxidative stress. Higher rates of instantaneous mortality is another potential cost of rapid growth. In three-spined stickleback fish, rapid growth has been shown to also reduce the lifespan through rapid skeletal growth (Inness and Metcalfe, 2008).

There are environmental constraints during growth that can also impact the pattern of growth with immediate and long-term impacts for animals. Food deprivation is a prime example of this (Moe et al., 2004) but stress is a further example. When an individual experiences a stressor, glucocorticoid hormones, such as cortisol, and corticosterone (dependent on species), are released to facilitate the response to the stressor and then to return the body to baseline function. Yet, if an individual receives prolonged or recurrent stress exposure this can disrupt homeostasis, leading to stress-induced diseases (Romero and Beattie, 2022). Stress has been linked with both weight loss and weight gain in a variety of taxa; mammals (Kannan et al., 2000; Reeder and Kramer, 2005; Schuamann et al., 2014; Kershaw and Hall., 2016, Malik and Spencer, 2019), birds (Moe et al., 2004; Dickens et al., 2009), amphibians (Crespi and Denver, 2004), and reptiles (Dunlap, 1995). It has been noted that early life stress, including maternal gestational and juvenile stress, can cause weight gain as it can alter feeding and metabolism (Malik and Spencer, 2019). In female sheep, when corticosteroids are given at the end stage of pregnancy, it leads to increased weight and fat masses in the adult offspring (Berry et al., 2013). Repeated exposure to stressors in late pregnancy can also lead to metabolic vulnerability to a high fat or a high fat-high sugar diet in adult offspring (Malik and Spencer, 2019). Malik and Spencer (2019) further suggested that parental or combined parental influence is important in the programming of metabolic and hypothalamus-pituitary-adrenal (HPA) axis outcomes. Early life stress in juveniles can cause alterations to the limbic system. In rats, early life stress can cause juveniles to perform poorly in spatial learning tasks (Avital et al., 2006). In rodent pups, when the dam alters her behaviour due to a stressor (e.g., the loss of nesting material (Ivy et al., 2008)), the stressor leads to detrimental long-term metabolic outcomes of the pup even with access to an adequate milk supply from the dam. The pups exhibit persistent

reductions in white adipose tissue mass but experience long-term weight regulation difficulties with increased weight gain and body fat when exposed to unhealthy diets later in life (Yam et al., 2017). Within marine mammals, there is further evidence of parental stress exposure impacting offspring weight gain through glucocorticoids passing through lactation (Stead et al., 2021; Armstrong et al., 2023).

Stress can have a direct effect on food intake, i.e., weight, but it can also have indirect effects through energetic costs, i.e., diversion of resources. Stress-exposed individuals can respond by increased food intake hence weight gain or through appetite suppression hence weight loss (Reeder and Kramer, 2005; Malik and Spencer, 2019), seen in many species such as fish (Bernier, 2006), toads (Crespi and Denver, 2004), and mammals (Harris et al., 1998; Maniam and Morris, 2012). Another way stress can affect weight is through time budget and energetic costs of lower rest and heightened vigilance. Rest is involved in the regulation of body weight and the amount of rest and synchronisation of the biological clock are necessary for energy balance and the secretion of hormones that contribute to weight regulation (Leger et al., 2015). Sleep deprivation have been shown to cause weight change in both directions. In humans, it has been shown to cause weight gain through increased food intake (Chaput and Tremblay, 2012), but in mice, sleep deprivation is associated with weight loss as the amount of food eaten during sleep deprivation was insufficient to cover the energy expenditure further leading to a metabolic deficit (Đukanović et al., 2022). Complete rest is important, but fragmented sleep, in humans, has been associated with impaired alertness, memory, mood regulation, changes to brain activity and metabolism (Short and Banks, 2013).

Environmental stressors, such as physical disturbance, can also play a part in regulating behaviour. Social and physical disturbance has been shown to alter the behaviour of Australian field crickets (*Teleogryllus oceanicus*). Rudin et al., (2018) found that individuals switched from a silent environment to an acoustic environment became less bold, active, and explorative. However, when moved from an undisturbed to disturbed environment, the crickets only became less explorative, indicating that changes within the social environment had a greater effect. In meerkats (*Suricata suricatta*), there is some evidence that an increase in vigilance may occur due to previously experienced stressors through an increase in glucocorticoids, potentially causing weight loss (Voellmy et al., 2014). Evidence of these behaviours and how they might be impacting weight, are important welfare indicators that may be particularly relevant in the rehabilitation of grey seals.

1.2: Grey Seal Phenology:

There are three stages in the annual cycle of the eastern north Atlantic grey seal: breeding season (August to December), moulting (December to April), and a period of time feeding and hauled out either on land, ice or at sea (Lidgard, 2003; In the UK, these are public available records from North Wales Wildlife Trust, 2021a, 2021b, 2021c). Female grey seals become sexually mature at five years old, reproducing annually. At most breeding sites, there are overlapping generations as grey seals are heroparous capital breeders with a high degree of site fidelity (Bull et al., 2017). Females give birth to one pup a year weighing 10 – 15kgs on average (Based on publicly available records from Cumbria Wildlife Trust, 2023). Grey seal pups progress through four different phases before becoming a juvenile (Table 1). Grey seal pups are not water resistant at birth and are unable to swim due to the lanugo (white) coat. Grey seals have a three-

week nursing period, where for the first 16 – 20 days, they rely solely upon the mother's milk to build up strength and fat reserves (feeding up to six times a day) (Based on public available records from Cumbria Wildlife Trust, 2023). The milk transferred from the mother to the pup contains fat (50%), protein (12%), and water (36.2%) (Baker, 1990). During the lactation period, female grey seals can lose 84% of stored energy reserves, with the pup gaining 46% of the mass lost by the mother (Reidman, 1990a). At three weeks, the pup is abandoned by the mother. The pup weighs between 30 and 40kgs and relies upon the weight gained through the learning phase (learns to hunt and swim). During this phase, the seal relies upon its fat reserves, so the larger the fat reserves, the better the chance the seal pup has to survive (Hall et al., 2008). The diet post-weaning mainly consists of fish such as salmon, cod, herring, mackerel, cephalopods (such as squid), and crustaceans (Reidman, 1990b).

Wild grey seals also do not follow a linear weight trajectory throughout their lifespan (Figure 1) due to important life history processes and seasonal environmental dynamics such as reproduction and moulting (Figure 1C) (Silva et al., 2020). Pup growth is faster during the weaning period before decreasing post-weaning, before slowly increasing again (Figure 1B). Weight gain decreases post-weaning due to the required foraging skill acquisition, where pups rely solely upon the fat reserves built up during weaning. In captive grey seals, growth is much slower, gradually catching up to their wild counterparts (Figure 2).
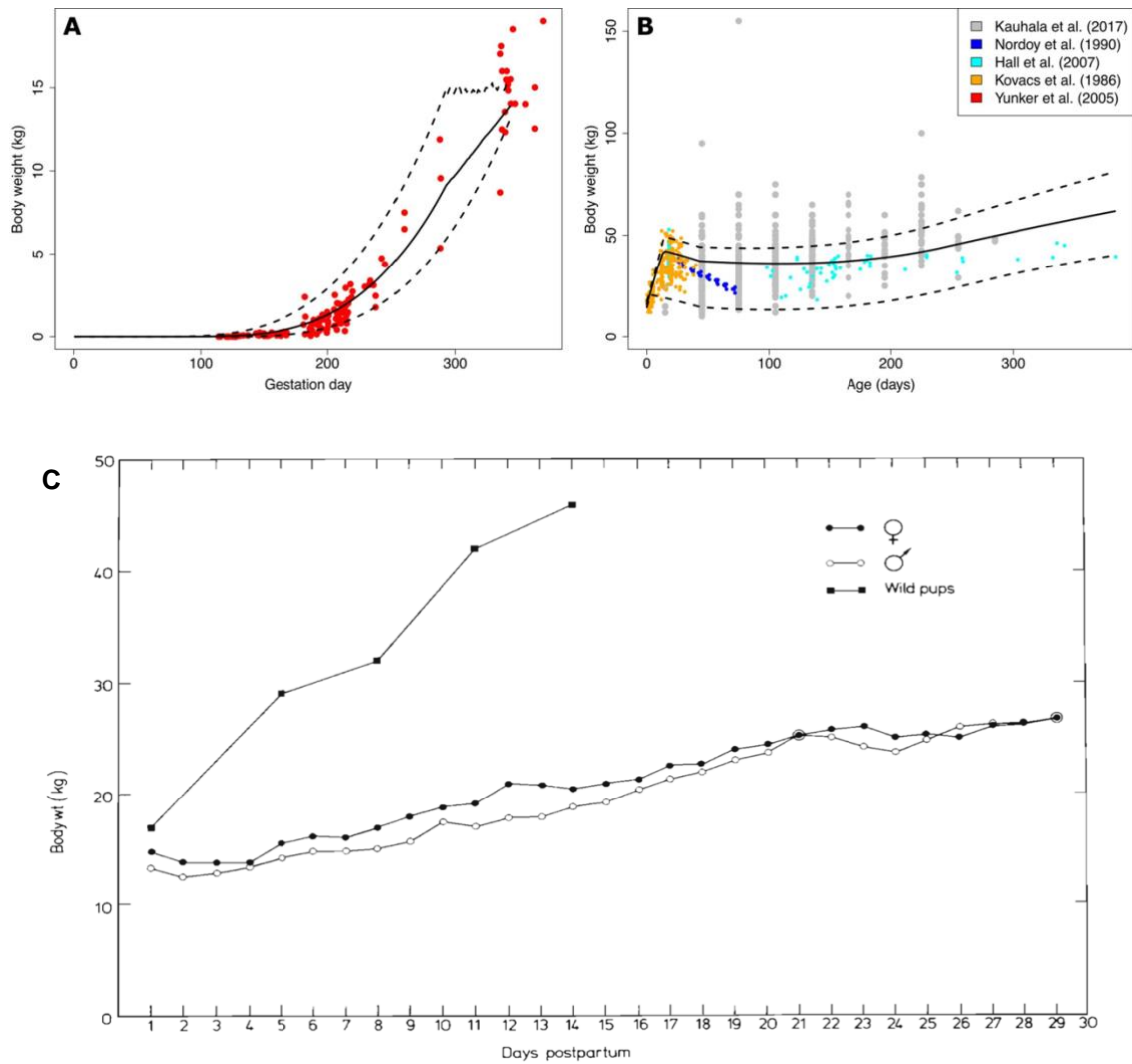
Figure 1: Adapted from Silva et al., 2020, and Spotte and Stake, 1982. Simulated growth data from a lifecycle dynamic energy budget (DEB) model vs. empirical observations of female grey seal body growth. (A) Body weight during foetal growth. (B) Body weight of weaning and juveniles. Points represent empirical data from five studies, solid lines represent the mean simulated body growth and dashed lined represent the range (min – max) of the simulated data (Silva et al., 2020). (C) Weight gain of two captive-reared grey seal (*Halichoerus grypus*) pups, compared with wild pups on Sable Island, Nova Scotia (Spotte and Stake, 1982).

Table 1: The natural progression of grey seals (*Halichoerus grypus*) from birth to adulthood. Age classes are a functional way of aging pups from within each pupping season. In southwest England, pups appear to complete the moult before weaning (Barnett et al., 2000). Table based upon data gathered from Barnett et al., (2000) and Zatrak et al., (2022). These age classes are also applied within rehabilitation centres.

| Age Class | Phase | Age | Description |
|---|---|---|---|
| | | 0 – 2 days | Umbilicus is pink, moist, and bloody. Neonatal lanugo coat is present. |
| | | 3 – 4 days | Umbilicus is pink and dry at the top. Neonatal lanugo coat is present. |

| | | | |
|---|---|---|---|
| *Neonate* | **Phase 1** | 5 – 6 days | Umbilicus is dry, shrivelled, and grey/black in colour. Neonatal lanugo coat present. |
| *White-coat* | **Phase 2** | 7 – 14 days | Umbilicus lost, neonatal lanugo coat present. |
| *Mid-moults* | **Phase 3** | 11 - 16 days | Lanugo coat partially lost. |
| *Moulted* | **Phase 4** | 11 days – 10 months old | Lanugo coat lost. 16 – 21 days weaning occurs. At 21 days, mother abandons the pup and learning phase commences. |
| *Juveniles* | | 1 – 2 years | During second moult, fur loses structure and becomes orangey brown. Remain solitary or with other juveniles. |
| *Adolescent* | | 3 – 5 years | Behaviour is learned though interactions with adults and other adolescents. |
| *Adult* | | >5 years | Reached full size and weight. Females start breeding between five and six. |

Phocid pups in general have a high first year mortality rate (Hall et al., 2002; Harding et al., 2005; Hall et al., 2008; Jenssen et al., 2010), with 43% of juvenile grey seals reported in the Netherlands over a 30-year period (1988 and 2002 excluded due to high mortality caused by phocine distemper virus *(Phocine morbillivirus)* epidemic) (Osinga et al., 2012). There is evidence of a positive correlation between weight at weaning and survival post-weaning (Hall et al., 2008; Jenssen et al., 2010), potentially stronger in male pups (Hall et al., 2008). At two breeding sites in the U.K., the primary cause of pup mortality was starvation (19.6%), but other causes included septicaemia (14%), peritonitis (12%), and drowning (12%) (Anderson et al., 1979). There is further evidence of a wider range of anthropogenic effects on grey seals, including entanglement within fishing lines and bycatch (Sayer et al., 2021). During a 10-year period (2010 – 2020), 81.4% of grey seals were reported as entangled, with young grey seals the most

susceptible (Salazar-Casals et al., 2022). Salazar-Casals et al., (2022) reported that entanglements have gradually been increasing from 2017, with a spike after 2018. Other evidence has shown that maternal condition is a significant factor in the likelihood of pup survival. Mellish et al., (1999) found clear evidence that heavier females not only lactated for longer and had higher milk outputs, but also produced larger pups at weaning. Further evidence suggests that due to the increased frequency of severe winter storms within the last five years, 70% of seal pups die within their first year, with large numbers of pups being separated prematurely from their mothers (Jarvis and Marsh, 2017). Jarvis and Marsh (2017) further reported that wild grey seal pups were often underweight due to the inability to feed effectively in the post-weaning period.

1.3: Rehabilitation Centres:

Rehabilitation centres, or rescue centres, are centres that rescue and take in injured or ailing marine mammals such as seals, rehabilitate them and then release them back into the wild. Rehabilitation centres employ a variety of management practices to increase the likelihood of post-release survival and to reduce the impact on their welfare, including the replication of wild environment (e.g., enrichment, haul-out areas, access to water of varying depths), to meets the animals physiological and psychological needs, and the type of human interaction (British Veterinary Zoological Society, 2016). The majority of rehabilitation centres are designed to meet a minimum set of criteria in weight and health before release (Burroughes et al, 2021), but there are rehabilitation centres such as Seal Rescue Ireland that design practices to increase the likelihood of post-release survival. Most rehabilitation centres follow a set pathway; a hospital or intensive care unit (ICU) for initial care and treatment, a recovery area (sometimes referred to as Kennels), pools or enclosures for social and/or physical development, and

an area with reduced or removed human interaction pre-release. Wild animals enter rehabilitation centres for a variety of reasons, including injury, illness, entanglement (often in human made objects such as netting), and human disturbance. These reasons as well as the condition (e.g., weight) on entering the centre, can all impact management decisions as well as the likelihood of survival both pre- and post-release.

All rehabilitation centres have a large database of information recorded on individuals upon admittance. This information can be used for a variety of purposes, including tracking of populations, disease outbreaks (e.g., phocine distemper (*Morbillivirus*) outbreak in 2002 (Härkönen et al., 2006)), changes in admittance patterns for particular species (e.g., if there are more entanglement cases in one particular year) (Zatrak et al., 2022), reviewing management practices (Burroughes et al., 2021), and further factors that could be influencing survival. European hedgehogs (*Erinaceus europaeus*) are a great example as this species is frequently seen in rehabilitation centres; the Royal Society for the Prevention of Cruelty to Animals (RSPCA) saw over 19 thousand hedgehogs admitted over a 13-year period (Burroughes et al., 2021). Due to the plethora of data available from this period, it allowed for management care practices to be greatly improved (e.g., through increased experienced and expertise of rehabilitation staff), increasing the number of hedgehogs released (Burroughes et al., 2021). A survey by Guy et al., (2013) on current mammal rehabilitation and release practices, found that three primate rehabilitation centres reported habituation to humans as a potential problem. A very recent study on orphaned lynx in rehabilitation centres are another example as habituation to humans developed during the rehabilitation process, especially as they associated humans with food (Molinari-Jobin et al., 2024).

Although there is an extensive database available, in phocids, there is less research into management practices that could be influencing survival. If this data was harnessed, management decisions and practices would be better informed. Evidence of this has been seen in a recent study by Zatrak et al., (2022). This study evidenced that weight upon admittance of juvenile seals had a significant effect on survival; juvenile seals surviving to release increased by 1.07 times for every kilogram of weight over the predicted weight for their age. This was consistent with another study by Hall et al. (2002) where upon a heavier juvenile had higher survival probabilities. Zatrak et al., (2022) recommended that weight during the triage and treatment should be paid special attention to, making sure that individuals do not lose weight and therefore, decreasing their chance of survival. Interestingly, Zatrak et al., (2022) did also make another recommendation: more consistent record keeping and sharing of best practice guidelines among seal rehabilitators, to further enable research into admission reasons and survival probabilities of admitted seals.

It is estimated that 40% of the world's population of grey seals are in UK and Ireland (Zatrak et al., 2022). Within the U.K. and Ireland, there are 13 specialist seal rescue centres and hospitals, and the British Divers Marine Life Rescue (BDMLR) reported that the total rehabilitation capacity within the U.K. often reached its limit during peak pupping season, due to severe storms, reduced ability to feed effectively post-weaning, and an increase in human disturbance (Jarvis and Marsh, 2017). In a recent review of grey seal and harbour seal admittances between 1988 and 2020 and 4126 individuals (2691 grey seals and 1435 harbour seals) over five rehabilitation centres (Zatrak et al., 2022), the most common nonexclusive reasons have included: malnourishment (37%), injuries (37%), maternal abandonment pre-weaning (15%), lethargy (12%), and parasite infections (8%). Other evidence suggests that the most common presenting condition is

trauma: in a review of 205 seals admitted between 2005-2011, Silpa et al. (2015) found that 81.95% of pups also exhibited at least one form of trauma: puncture trauma (68.78%), abrasions (36.59%), orthopaedic disorders (11.7%), and netting injuries (3.4%). These trauma injuries further can lead to other health conditions developing including malnourishment and hypothermia.

Weight-gain is crucial for an individual's health, but it can also be indicative of success upon release. In the wild, grey seal pups gain weight rapidly (Figure 1B), but individuals within rehabilitation centres and captivity grow and gain weight at a lower rate (Figure 1C)). O'Hara (2019) found that grey seals pups in rehabilitation gained weight 7.7% of the mean daily weight that individuals in the wild were gaining However, weight-gain is not always easily attainable within rehabilitation centres, as, for example, replicating the milk for pre-weaning pups is difficult (MacRae et al., 2011). A common feeding method used for seals is gavage feeding (an immediate feeding method, involving restraint as a tube is forced into the oesophagus). However, this feeding method can trigger the stress response which halts the digestive process, further slowing the weight gain of individuals (MacRae et al., 2011). This has been evidenced in harbour seals; MacRae et al. (2011) found that individuals fed on milk-replacers had higher weight gain and a lower mortality rate compared with a fish formula (a common feeding practice within rehabilitation centres due to cost of milk replacement). Rehabilitation centres have tried to replicate the milk with some success (MacRae et al., 2011). Typical hand-rearing diets include artificial milk-replacers and diets based on macerated fish that is fed via gavage, but often weight gain is slow. It is also important that individuals increase in weight rapidly to reduce the time spent in the centres and further risk of habituation to humans (Cope et al., 2022). There is evidence that habituation to humans happens in a wide variety of taxa in rehabilitation centres (Molinari-Jobin et al., 2024). Pups at post-

weaning are then fed a different diet comprising of deceased whole herring. This is to both replicate the natural progression of diet, and some aspects of behaviour such as food handling, of seal pups in the wild, but also herring is an easily accessible and cost-effective product.

Stressors associated with weight change in experimental research are often similar to management practices within rehabilitation centres (e.g., handling (Harris et al., 1998), transportation (Kannen et al., 2000; Schuamann et al., 2014), changes in social grouping (McLaren et al., 2023), disturbance (Jayakody et al., 2008)). Recent evidence has shown, for example, that handling of wood mice (*Apodemus sylvaticus*) in a captive breeding context causes weight loss, with intensive handling having greater weight loss (McLaren et al., 2023). Evidence has also shown that changes in social grouping can cause stress (indicated through physiological and behavioural indices) within social species such as bottlenose dolphins (*Tursiops truncatus*) and can cause weight loss in captive individuals (Waples and Gales, 2002). Environmental stressors are also known to obstruct weight gain through stress-induced susceptibility to disease and can additionally impact their behaviour (Stead et al., 2021). Stress can alter feeding behaviours which can further cause weight loss (Maniam and Morris, 2012). In captivity, when an observer is present, as a potential source of disturbance, behaviours are known to change. A UK study saw resting and clustering behaviours in captive breeding Edwards' pheasants significantly increase when an observer was present but feeding and locomotion behaviours significantly decreased (Hoy and Brereton, 2022). Human disturbance in captive management of wild species is also associated with increased wariness (Merrick and Koprowski, 2017), for example increased vigilance wild red deer (*Cervus elaphus*) (Jayakody et al., 2008).

All rehabilitation centres have a variety of pools that the pup passes through once they meet a set criteria (which can vary slightly from centre to centre, this is illustrated below for Seal Rescue Ireland (Figure 2)) that can include a certain weight, completion of treatment or loss of disease signs, recovered from injury, and are able to feed on their own (based on publicly available records from Cornish Seal Sanctuary, 2024). There are optimal paths based on design features, but in practice, management decisions are affected by the capacity of the centre or by disease quarantine (Figure 3). The design of pools are intended to provide opportunities for development of vital skills (e.g., swimming, diving, social skills) and physiological attributes (e.g., muscle mass through exercise), that may support survival in the wild. Rehabilitation centres have a goal release weight which replicates their wild counterparts (typical weight of weaned wild pups is 35 – 40kgs in grey seals) (Based upon publicly available data from Cornish Seal Sanctuary, 2020). Once they have reached this weight, pups are then released back into the wild.
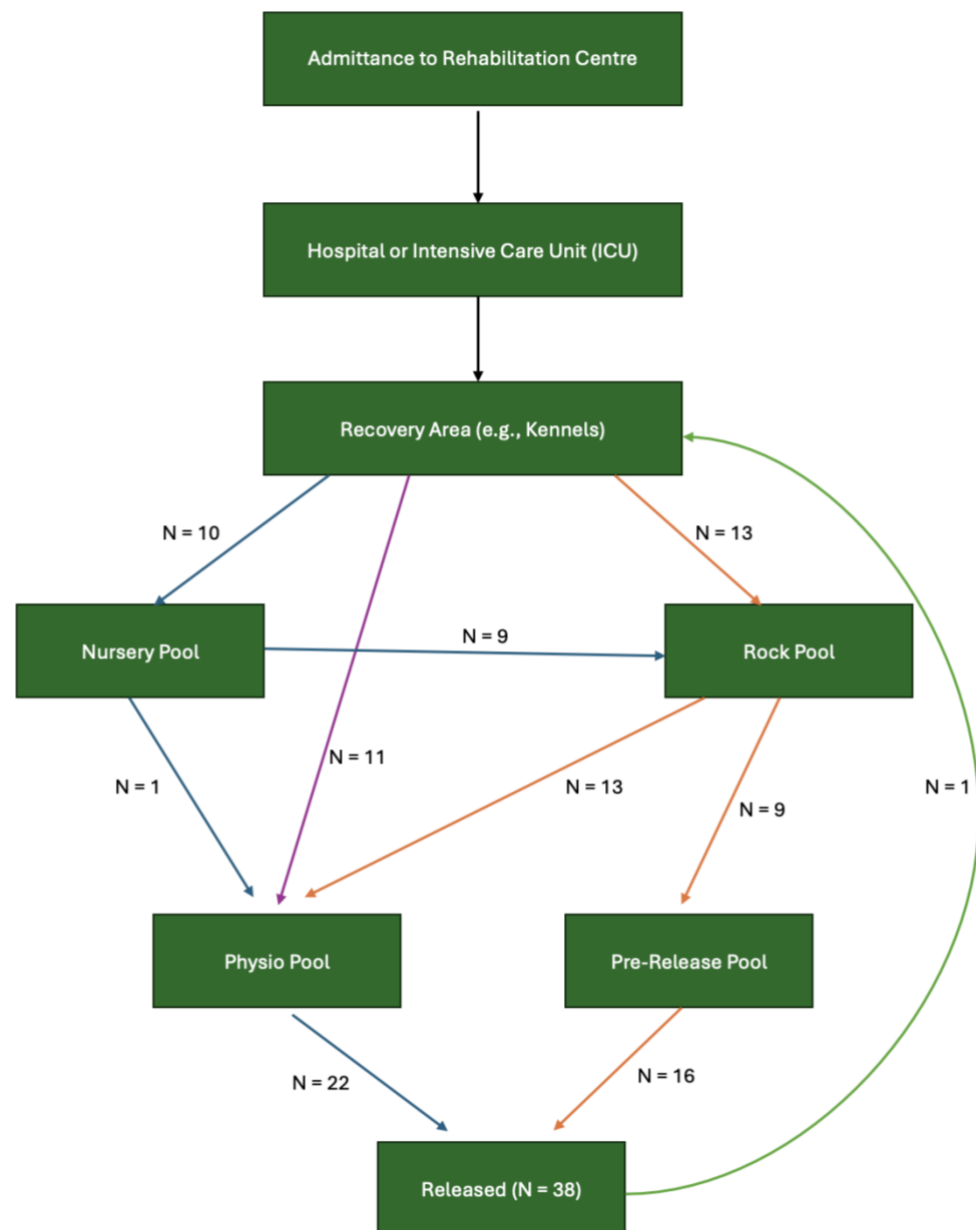
Figure 2: Seal rehabilitation process example; Seal Rescue Ireland (S.R.I.), Courtown, Wexford, Ireland. This a sample of the pupping season 2022 – 2023 (a total of 38 individuals were released). When a seal pup is admitted to the centre, it follows a pathway. First, the seal goes to the Intensive Care Unit (ICU) or Hospital, to receive immediate treatment and observation. From here, they go into a recovery area, also known as kennels at the S.R.I. The individual is then moved into one of the three pools (pathways blue, purple, and orange) dependent on management factors such as social structure, weight, and health. Within each of the different stages the number in brackets represents the number of individuals moving between each section in the 2022 – 2023 pupping season at the S.R.I. On the odd occasion, individuals can sometimes return to the kennels due to, e.g., the development of an injury (N=1 in 2022 – 2023 pupping season sample (green pathway)). They progress through the system of pool before reaching the final two: Physio and Pre-Release Pool. Once they have reached the target weight of 35 – 40kgs, individuals are then released (usually with a few individuals from the final social group).

1.4: This Study:

Common rehabilitation practices (e.g., change in environment (Stead et al., 2021), changes in social grouping (Waples and Gales, 2002), and disturbance (Jayakody et al., 2008; Rudin et al., 2018; Hoy and Brereton, 2022)), are known to cause stress to individuals in other contexts. Yet, within a rehabilitation context, the impacts of these stressors on grey seal pup weight gain and survival are less known. These practices are expected to have certain behavioural effects that should also be measurable. Therefore, it is important to measure factors such as these and how they impact growth, behaviour, and success of individuals, to better inform management practices.

There are factors outside of the rehabilitation centres control such as the admittance reason, sex, intake weight, and pupping year, that have already been evidenced to impact survivorship (as seen by Zatrak et al., 2022). This study aims to see if there are management practices inside the rehabilitation centres' control, such as movement between pools (e.g., number of pool changes and the pool characteristics) and natural disturbances (e.g., presence of wildlife (cause for sudden disturbance (Gaspari, 1994)), that could further be impacting weight gain, mortality, and the behaviour of grey seal pups alongside those key factors previously identified within a rehabilitation setting over a six-year period. The objectives of this study are as follows:

- To investigate the effect of known management practices on weight gain (N = 292 grey seals) using a linear regression analysis and mortality (N = 286 grey seals) using a generalised logistic regression over a six-year period.
- To Investigate the effect of unknown management practices (i.e., pool movement) between pools on weight and mortality of individuals over one pupping season through a repeated measures regression analysis of <70

individuals capturing effects of the pool changes. I hypothesise that given associations between changes in context and stress, I predicted that the higher the number of pool movements, the great the effect on weight gain and mortality.

- To investigate the impact of specific management practices on behaviour (specifically rest, vigilance, and movement) between each pool through a regression analysis. Repeatability, as described by Dingemanse et al. (2010), was also used to account for repeated measures on individuals to look at pool effects. I hypothesise that grey seal behaviour could be impacted by management practices currently in place, such as water level and pool movement. I further hypothesise that these behaviours are expected to indicate either stress or disturbance hence an effect on growth and mortality.

## 2: Methodology:

Data was collected from a rehabilitation centre in Ireland; Seal Rescue Ireland (S.R.I.), Courtown, Wexford, Ireland. The S.R.I. have a rehabilitation system involving an ICU unit, kennels, and four separate pools. These pool systems are designed to allow for the development of physical attributes and for natural competition between individuals, preparing the seal pups for release. The S.R.I provided weight and mortality data in a raw format (.csv files), exported straight from their Salesforce Lite database. Individual data collection from the raw format to understand management effects on weight (Seal pupping years 2018 ± 2024, N weight measurements = 3433, N individuals = 292) and mortality (Seal pupping years 2018 ± 2024, N mortality measurements = 286 (26 mortalities)). Due to a malfunction on the S.R.I.'s Salesforce Lite software, data had been lost for pool movements pre-2022. To understand management effects, i.e., pool, on weight, a subset of individual data was collected from the raw format files for seal pupping year 2022-2023 only (Seal pupping year 2022 – 2023, N weight measurements = 368, N individuals = 67; N mortality measurements = 69). Individual behaviour data was collected on a subset of individuals (pupping year 2022 – 2023, N behavioural samples = 413, N individuals = 31), through Reolink Go PT Plus cameras (Reolink, China) placed by each of the four pools, to understand pool effects on behaviour.

## 2.1: Rehabilitation Centre:

The S.R.I. site is made up of a visitor centre, an intensive care unit (ICU), kennels, and four separate pools: Nursery, Rock, Physio, and Pre-Release (Figure 4 and Table 2). The water is from a local freshwater source and is filtered as pools are refilled. Water drains from each of the pools allowing for cleaning of the pools base. The Pre-Release pool has a small section within the pool area where there is still accessible water during drainage.

The number of grey seals within the centre changes continually between August and March with new intakes and releases.



Figure 3: Aerial view of the Seal Rescue Ireland site, Courtown, Wexford, Ireland (Duncan Kenny Drone photo). Locations of Reolink Go PT Plus (Reolink, China) cameras are indicated. See Table 2 for specific pool details.

Table 2: Attributes for four pools at Seal Rescue Ireland, Courtown, Wexford, Ireland.

| Pool | Pool Attributes | Pool Measurements |
|---|---|---|
| Nursery Pool | Smaller and shallower than other pools. Primary use is for seals coming out of ICU/ Kennels. | Length: 10.5m. Width largest point: 7m. Width smallest point: 4m. Pool depth (deepest point): 1m. Pool volume estimate: 38 – 40000L. |
| Rock Pool | Larger and deeper than Nursery Pool (suited for pups further along the process). | Length: 17.5m. Width largest point: 8.5m. Width smallest point: 6m. Pool depth (deepest point): 1.5m. Pool volume estimate: 115000L. |
| Physio Pool | Larger and deeper than Nursery Pool (suited for pups further along the process). Section within pool that requires seals to climb over it; intended to develop skills and essential physical attributes. | Length: 15m. Width largest point: 8m. Width smallest point: 6m. Pool depth (deepest point): 1.5m. Pool volume estimate: 95000L. |

| | | |
|---|---|---|
| *Pre-Release Pool* | Largest pool and final stage of rehabilitation. Pool is enclosed with tall fencing designed to minimise human interaction for the final few weeks before release. Small section within pool area where water is still accessible during drainage. | Length: 20m. Width largest point: 11.5m. Width smallest point: 6m. Pool depth (deepest point): 1.5m. Pool volume estimate: 155000L. |

2.2: Weight and Mortality:

The following information for 466 seals from 2012 to 2024 was collated from Salesforce Lite, via manual collation from database and notes provided by the S.R.I.: field identification number (Field ID), sex (male/female), age class (1-3 days, 4-7 days, 7-14 days, 2-4 weeks, 4-6 weeks, 4-8 weeks, 2-4 months, 4-6 months, 4-8 months, 6-12 months, unknown), intake weight (kgs), actual weights (kgs), date of measure, admission reason (emaciated, underweight, illness, injured, dehydrated, orphaned, premature, entanglement, bad location, harassed, and unknown (see Supplementary 1)), location (ICU, Kennels, Nursery Pool, Rock Pool, Physio Pool, and Pre-Release Pool), date moved, mortality (released/deceased), decease date, cause of death if known, pupping year, and additional comments.

Three individuals were excluded because there were records for less than two individuals in their pupping years, compared to 2019 ± 2024 otherwise, so management may not be comparable. 139 individuals were excluded because they had fewer than four recorded weights (threshold was chosen due to malfunctions with the weighing scales upon admittance where some weights were repeated). 26 individuals were excluded because they were currently in the rehabilitation centre at the time of analysis (i.e. outcome unknown). Four individuals were excluded due to unknown age class. Two individuals were re-admitted, for which only data from the first admittance was included. Given the inconsistency in reporting and increasing uncertainty with

increasing age class, age class was conservatively modified into dependent (still reliant on the mother (0 – 4 weeks), N individuals = 55, range: 9.2 – 33.4kgs, average: 15.71kgs) and non-dependent (not reliant on the mother (4+ weeks), N individuals = 239, range: 9.4 – 37.45kgs, average: 16.86kgs). Two individuals of age classes six to 12 months were removed because it is unknown where individuals are in linear growth phase (approx. 200 to 400 days (Figure 1)).

The duration of time spent in the rehabilitation centre from initial intake was calculated for each weight (Date measured – Intake date). Weight change between days was calculated per individual by (mean: 0.25kgs, std. dev: 0.38):

$$\frac{Wd}{ND}$$

Where *Wd* = Weight difference between each weight measurement (mean: 1.76kgs, std. dev: 2.18) and *ND* = Number of days between each weight measurements (mean: 6.27 days, std. dev: 3.07).

Due to a malfunction on the S.R.I.'s Salesforce Lite software, data had been lost for pool movements pre-2022. To understand management effects, i.e., pool, on weight, a subset of individual data was collected from the raw format files for seal pupping year 2022-2023 only. Therefore, final sample sizes for weight were N individuals = 292, N weight measurements = 3433 for years 2018 – 2024, and N individuals = 67, N weight measurements = 378 for year 2022 – 2023.

Mortality (0 = alive, 1 = deceased) during rehabilitation was censored to <40 days in rehabilitation (threshold set at 40 days as average death occurred at 40 days). Weight change for analysis of predictors of mortality (mean: 8.92kgs, std. dev: 4.8) was calculated by actual weight (kgs) at end of 40-day period (or closest final weight to this) – intake weight (kgs). Total duration of rehabilitation was noted for all individuals within this analysis (numbers of days between intake date and release/decease date). Due to

the aforementioned issue on the S.R.I's software, data had been lost for pool movements pre-2022. To understand management effects, i.e., pool, on mortality, a subset of individual data was collected from the raw format files for seal pupping year 2022-2023 only. Number of pool moves was censored to <40 days (mean: 1.04 moves, std. dev: 0.85). Final sample sizes for mortality were therefore N measurements = 292 for years 2018 – 2023 and N measurements = 72 for year 2022 – 2023.

2.3 Behaviour:

Behavioural data was collected on 39 seals on eight days between 14th February 2023 and 16th March 2023. All seal pups were moved only according to S.R.I. practice including for medical treatment, weighing, changing pools, or release when they reached the desired weight of at least 35kgs. The behavioural data was collected using a Reolink Go PT Plus camera (Reolink, China) stationed at each pools (Figure 4, see Supplementary materials 2 for further details). The cameras recorded onto 32GB SD cards. Cameras recorded on a 30 second interval timelapse between 00:00:00 and 23:59:59 on a three-day rotation, recording through infrared at night.

Due to the extensive data (n = 32873 snapshots) and camera malfunctions/faults, behavioural data was only collected between 12:00 and 16:00 at 5-minute intervals during the day (collected by myself and a second observer, n = 768; henceforth 'day data') and in three-time categories during the night (collected by myself): 00:00 – 01:00 (n = 2400), 02:00 – 04:00 (n = 720), 05:00 – 07:00 (n = 1440; henceforth 'night data'). A combined dataset was compiled using both night and day data (day: n = 2102, night: n = 910; N = 31; henceforth 'combined data'). Individual ID was collected for 95% of individuals within images using an ID guide that I created from photos collated by the S.R.I. upon instruction. Identification was based on pelage markings, head shape, visible

injures, colouration caused by treatments (Beaumont and Goold, 2007; In the UK, these are public available records from Dorset Wildlife Trust, 2023). All unidentifiable individuals were removed from the analysis.

Four non-mutually exclusive behaviours were collected for both day and night data: "head elevation", "movement", "resting", and "aggression" (Table 3). These four behaviours were selected through a pilot study where I added behaviours as they were observed in 240h timelapse in one pool to construct an expanded ethogram, to identify common behaviours that could be objectively classified (unlike e.g., distance of proximity to another seal)), and was informed by some studies on wild grey seals (Chilvers et al., 1999; Twiss and Franklin, 2010). These four behaviours are biologically important to link weight gain and stress. Head elevated was included with the intention of capturing alertness or vigilance or may equally capture neutral or positive interactions, e.g., social or feeding opportunities.

Table 3: Behavioural ethogram created for data collection on time-lapse images of seals.

| Behaviour | Definition |
|---|---|
| Head elevated | Head is up from head down position. Individual's head can be slightly raised from head down position or raise significantly with neck extended. |
| Movement | Any movement from original position that is active and require energy expenditure. This can include one of the following: swimming (both at surface and underwater), looking underwater, galumphing (moving in a humped motion forward), climbing, scratching. Can coincide with head elevated behaviour. |
| Rest | Head down, eyes can be open or closed. Can be in the water or on land. Includes bottling and logging behaviour seen within the footage (Seals are resting but are able to surface and go back underwater without waking, eyes remained closed, head can be in a particular direction. Logging is horizontal, bottling is vertical). Can coincide with elevated behaviour. |

| | |
|---|---|
| *Aggression* | Individuals within a close distance, teeth can be visible, neck forward, lips raised. Two individuals face on, nose to nose (with muzzles touching). Can be seal directed, human (staff) directed, or wildlife (e.g., heron) directed. |
| | Can coincide with head elevated and movement behaviour. |

Contextual variables were recorded from the snapshots to capture disturbances, social factors and environmental factors that may affect behaviour on the specific individual. During pilot data collection, only herons were observed to alter seal behaviour (seal pups were observed chasing herons) in comparison to other wildlife present (e.g., sea birds) so was included within the data collection.

Table 4: Contextual variables considered to affect the behaviour of individuals across pools.

| Environmental Factor | Description: |
|---|---|
| *In Proximity* | 0 = not in proximity. 1 = within a seal body length of another individual. |
| *Water Level* | 0 = little water to no water, 1 = Shoulder depth, 2 = swimmable/full |
| *Intensity of people* | 0: No people in the pool. |
| | 1 (Low Intensity): People in enclosure, seals not reactive. Staff very briefly in pool (less than five minutes recorded via timestamp in snapshots). |
| | 2 (Medium intensity): People in enclosure, seals are reactive, but staff are not interacting with seals. Staff are in pool for short period of time or an extended period of time but no disturbance to seals, e.g. pool clean (over 10 minutes recorded via timestamp in snapshots). |
| | 3 (High intensity): People in enclosure. Staff are interacting with seals (e.g., weighing, medication administration). |
| *Staff Feed* | Staff are throwing fish into pool. This occurs between two and three times at varied times throughout the day between 07:00 and 19:00 during the observation periods. |
| *Weather* | Rain versus dry, visible in image. |
| *Heron* | 0: Absent from the pool. 1: Present in the pool. |

2.4: Data Analyses:

Data was analysed using R (version 4.3.1, (R Core Team, 2023)) via the software R Studio, version 2023.12.0+369 (R Core Team, 2023). All visual outputs have been produced through "sjPlot" (Lüdecke, 2023) and "tidyverse" (Wickham et al., 2019).

2.4.1: Weight Change:

Weight change was analysed using linear mixed effects models (LMER's) for six consecutive over-winter pupping seasons between 2018-2024 (N individuals = 292, n weight measurements = 3433), using the package "lme4" (Bates et al., 2015). Weight change between consecutive measurements (kgs) was the dependent variable instead of actual weight per measurement to increase sensitivity for capturing effects of current context. Overall weight and age might both affect capacity to change weight, were controlled for by including actual weight as an independent variable. Further fixed independent variables were duration of rehabilitation to date of weighing (days), sex (male/female), age (Dependent or Non-Dependent), and admittance reasons: underweight (yes/no), illness (yes/no), injured (yes/no), and seal pupping season (2018 – 2019 (n = 192, N individuals = 10), 2019 – 2020 (n = 877, N individuals = 73), 2020 – 2021 (n = 924, N individuals = 59), 2021 – 2022 (n = 619, N individuals = 48), 2022 – 2023 (n = 571, N individuals = 72), 2023 – 2024 (n = 70, N individuals = 10)). Individual ID (Field ID) was controlled for as a random effect in the analysis.

As pool data was only available for 2022 – 2023 seal pupping season, a separate analysis was done for weight. The same fixed independent variables and random effects were considered with the addition of pool ID at measurements, and if they changed pool between measurements (yes/no). ICU and Kennel were not included in the pool

movement as the focus was specifically on the pools. Changes included movement from kennel to pool to include the first weight.

2.4.2: Mortality

Mortality (0 = "alive", 1 = "deceased") was analysed using a generalised logistic regression model (GLM, family = binomial) for the same six consecutive over-winter pupping seasons (2018-2024) (N = 292), through package "lme4" (Bates et al., 2015). There were 26 mortalities (including individuals euthanised (n = 7 seals)) and 268 released. Fixed independent variables were age (Dependent or Non-Dependent), sex (male/female), intake weight (kgs), weight change at 40 days, standard deviation of weight change (0 – 40 days) (as a measure of consistency in expected weight gain), duration of rehabilitation (total time), admission reasons: underweight (yes/no), injured (yes/no), and illness (yes/no). Seal pupping season was removed due to uneven mortalities and limited data within some years to explore mortality (2018 – 2019, 10 data points, 0 mortalities).

Pool effects on mortality were analysed for 2022 – 2023 pupping season by including number of pool moves in the first 40 days as an independent variable (N = 72 of which seven were mortalities (including four euthanised)). ICU and Kennel were not counted as the focus was specifically on the pools. A further independent variable was added to the analysis which included the pool that was the most behaviourally distinct. As all seven mortalities were admitted with underweight as the reason, all admittance reasons were removed from this analysis.

2.4.3: Behaviour

Behaviour was analysed using generalised linear mixed effects models (GLMM's) on the combined data (n observations = 415, N individuals = 31), using package "lme4" (Bates

et al., 2015), for three behaviours (head elevated, movement, and rest). Aggression was not analysed due to rarity of behaviour (combined night and day: n observations = 28). Fixed independents variables were pool ID, time of day (day v. night), heron (absent v present), and in proximity of another seal. The reference levels were Pre-Release Pool, day, heron present, and not in proximity of another seal. Individual ID was included as a random effect to account for repeated measures of individuals, through comparing AIC numbers (see Supplementary 4 for further details). Confounding effects were tested for using package "car" (Fox and Weisberg, 2019). Observations of people and rain were confounded with day/night, so are not included in these models, but analyses for day and night data are presented separately in Supplementary materials 4.1 – 4.2.

The focus of the analyses was to explore pool effects on behaviour. To determine the extent to which behavioural variation observed was driven by individuals' consistency repeatability estimates, following Nakagawa and Schielzeth, 2010, using package "rptR" (Stoffel et al., 2017). Estimated adjusted repeatability (an estimate adjusting for confounding effects) was used as there were confounding effects within the GLMM's (a variable distorting the observed relationship between the independent and dependent variables). To be classed as repeatable, the estimates needed to be higher than 0.2 (Harper 1994).

2.5: Ethics

This study was approved by Seal Rescue Ireland and the University of Plymouth's School of Biological and Marine Sciences Animal Ethical Review Committee (2023). The study did not require any changes to normal management practices of the Seal Rescue Centre staff. As the cameras were facing away from the public, human ethical review was limited to notification of staff regarding timelapse recording, appropriate storage of

footage, and protocols to ensure personally identifiable information was omitted from

files shared by S.R.I. for analysis.

3. Results:

3.1: Weight

3.1.1: Variables Influencing Weight Change for Six Consecutive Pupping Seasons.

The actual weight (kgs) of an individual is positively correlated with weight gain between days (Table 5). The duration of rehabilitation is negatively correlated with weight gain between days (i.e., the loner the duration of rehabilitation, the lower the weight gain between days). Compared to individuals not underweight upon admittance, underweight individuals had greater weight gain between days. Compared to individuals without an illness, individuals admitted with an illness had lower weight gain between days. Compared to the 2018 – 2019 pupping season, 2019 – 2020, 2020 – 2021, and 2022 – 2023 pupping seasons all had greater weight gain between days. There was no effect of individuals on weight gain so management practices can continue without needing to account for any specific individual.

Table 5: Variables influencing weight gain over six consecutive pupping seasons (N = 292 seals, n = 3433 weight measurements). Reference level: dependent age (0 – 4 weeks); female, not underweight, no injury, no illness, 2018 – 2019 Seal Pupping Season. Random effects was individual ID (Field ID). Random effects: residual variance ($\sigma^2$) = 0.13, Between subject variance ($\tau_{00}$) = 0.00, Marginal $R^2$ = 0.097 Conditional $R^2$ = NA.

| Predictors | Estimates | Confidence Interval | p-value |
|---|---|---|---|
| (Intercept) | -0.29 | -0.37 – -0.20 | **<0.001** |
| Actual Weight (Kgs) | 0.02 | 0.02 – 0.02 | **<0.001** |
| Age: Non-dependent | -0.02 | -0.05 – 0.02 | 0.287 |
| Male | -0.01 | -0.04 – 0.01 | 0.251 |
| Duration of Rehabilitation | -0.00 | -0.00 – -0.00 | **<0.001** |
| Underweight | 0.10 | 0.07 – 0.14 | **<0.001** |
| Injured | 0.01 | -0.02 – 0.04 | 0.413 |
| Illness | -0.04 | -0.07 – -0.01 | **0.016** |
| 2019 – 2020 Seal Pupping Season | 0.07 | 0.01 – 0.13 | **0.014** |
| 2020 – 2021 Seal Pupping Season | 0.09 | 0.03 – 0.15 | **0.002** |
| 2021 – 2022 Seal Pupping Season | 0.06 | -0.00 – 0.012 | 0.059 |
| 2022 – 2023 Seal Pupping Season | 0.12 | 0.06 – 0.18 | **<0.001** |
| 2023 – 2024 Seal Pupping Season | 0.10 | -0.00 – 0.20 | 0.053 |

### 3.1.2: Variables Influencing Weight Change for Pupping Season 2022 – 2023.

The actual weight (kgs) of an individual is positively correlated with weight gain between days (Table 6). The duration of rehabilitation is negatively correlated with weight gain between days (i.e., the longer the duration of rehabilitation, the higher the weight gain between days). Compared to individuals without an injury, individuals admitted with an injury had higher weight gain between days. There was no effect of individuals on weight gain between days so management practices can continue without needing to account for any specific individual.

Table 6: Variables influencing weight change for pupping season 2022 – 2023 (N = 67 seals, n = 378 weight measurements). Reference level: dependent age (0 – 4 weeks), female, Pre-Release Pool, not changed pool, not underweight, no illness, and no injury. Random effects: individual ID (Field ID), residual variance ($\sigma^2$) = 0.15, Between subject variance ($\tau_{00}$) = 0.00, Marginal $R^2$ = 0.124, Conditional $R^2$ = NA.

| Predictors | Estimates | Confidence Interval | p-value |
|---|---|---|---|
| *(Intercept)* | -0.36 | -0.67 – -0.05 | **0.025** |
| *Actual Weight (Kgs)* | 0.03 | 0.02 – 0.04 | **<0.001** |
| *Age: Non-Dependent* | -0.06 | -0.17 – 0.05 | 0.321 |
| *Male* | -0.02 | -0.10 – 0.06 | 0.628 |
| *Duration of Rehabilitation* | -0.00 | -0.01 – -0.00 | **<0.001** |
| *Nursery Pool* | 0.11 | -0.07 – 0.30 | 0.227 |
| *Rock Pool* | 0.12 | -0.01 – 0.24 | 0.065 |
| *Physio Pool* | 0.01 | -0.09 – 0.10 | 0.901 |
| *Changed Pool (Yes)* | 0.02 | -0.07 – 0.12 | 0.634 |
| *Underweight* | 0.09 | -0.08 – 0.26 | 0.308 |
| *Illness* | -0.07 | -0.24 – 0.11 | 0.445 |
| *Injured* | 0.09 | 0.01 – 0.17 | **0.035** |

### 3.2: Mortality:

### 3.2.1: Variables Influencing Mortality for Six Consecutive Pupping Seasons.

There were no independent individuals impacting weight gain between days for all six consecutive pupping seasons (Table 7). There was no effect of individuals on mortality so management practices can continue without needing to account for any specific individual.

Table 7: Variables influencing mortality (0 = alive, 1 = death) for six consecutive pupping seasons (N = 286 measurements). Reference level: female, age dependent (0 – 4 weeks), not underweight, not injured, and no illness. $R^2$ Tjur = 0.059.

| Predictors | Estimates | Confidence Interval | p-value |
|---|---|---|---|
| (Intercept) | 3.65 | 0.19 – 77.24 | 0.396 |
| Male | 1.79 | 0.72 – 4.90 | 0.226 |
| Age: Non-Dependent | 0.54 | 0.21 – 1.47 | 0.207 |
| Intake Weight (Kgs) | 0.92 | 0.82 – 1.03 | 0.148 |
| Total Duration of Rehabilitation | 1.00 | 0.98 – 1.01 | 0.552 |
| Weight Change at 40 days | 0.97 | 0.88 – 1.07 | 0.555 |
| St.d. Deviation of Weight Change | 0.92 | 0.77 – 1.06 | 0.268 |
| Underweight | 0.33 | 0.20 – 1.13 | 0.068 |
| Injured | 0.51 | 0.20 – 1.24 | 0.147 |
| Illness | 1.24 | 0.40 – 3.50 | 0.692 |

3.2.2: Variables Influencing Mortality for Pupping Season 2022 – 2023.

Compared to dependent individuals, non-dependent individuals (4+ weeks) have a higher probability of mortality (Table 8). A high number of pool changes are associated with higher mortality.

Table 8: Variables Influencing Mortality for Pupping Season 2022 – 2023 (N = 72 measurements). Reference level: female, age dependent (0 – 4 weeks), not underweight, not injured, and no illness. $R^2$ Tjur = 0.348.

| Predictors | Estimates | Confidence Interval | p-value |
|---|---|---|---|
| (Intercept) | 0.06 | 0.00 – 63.57 | 0.412 |
| Male | 2.69 | 0.34 – 29.27 | 0.361 |
| Age: Non-dependent | 0.05 | 0.00 –0.72 | **0.044** |
| Intake Weight (Kgs) | 1.33 | 0.96 – 1.96 | 0.102 |
| Total Duration of Rehabilitation | 0.99 | 0.93 – 1.04 | 0.711 |
| Weight Change at 40 days | 0.93 | 0.72 - 1.19 | 0.576 |
| St.d Deviation of Weight Change | 1.01 | 0.57 – 1.57 | 0.957 |
| Number of Pool Movements | 0.11 | 0.01 – 0.59 | **0.028** |
| Behaviourally Distinct Pool (Rock Pool) | 0.69 | 0.02 – 13.91 | 0.812 |

3.3: Behaviour

There were significant differences in behaviour across pools. Head elevated also had a higher likelihood of being observing in Rock Pool when compared to Pre-Release pool (Figure 5a). Compared to Pre-Release Pool, there was a higher likelihood of observing movement in Rock Pool and Physio Pool (Figure 5b). Compared to Pre-Release Pool,

there was a lower likelihood of observing rest in Nursery Pool, Rock Pool, and Physio Pool, with Rock Pool being the least likely to observe resting behaviour (Figure 5c). When in close proximity to another seal, there was a lower likelihood of being observed in movement behaviour. When in close proximity to another seal, there was a higher likelihood of being observed in resting behaviour. Head elevated was also observed when herons were present. Compared to during the day, head elevated, and movement behaviour were less likely to be observed during the night. However, compared to during the day, rest was more likely to be observed during the night. The combined day-night results are presented for greatest utility to management (to allow for a general overview of changes within behaviour and pool effects), but when day and night were analysed separately, time of day and intensity of staff (i.e., medium) were also significant (Supplementary 4.3). All three behaviours scored lower than the threshold for repeatability ($R_M = 0.02$), but movement and rest were statistically significant (see Supplementary 4).
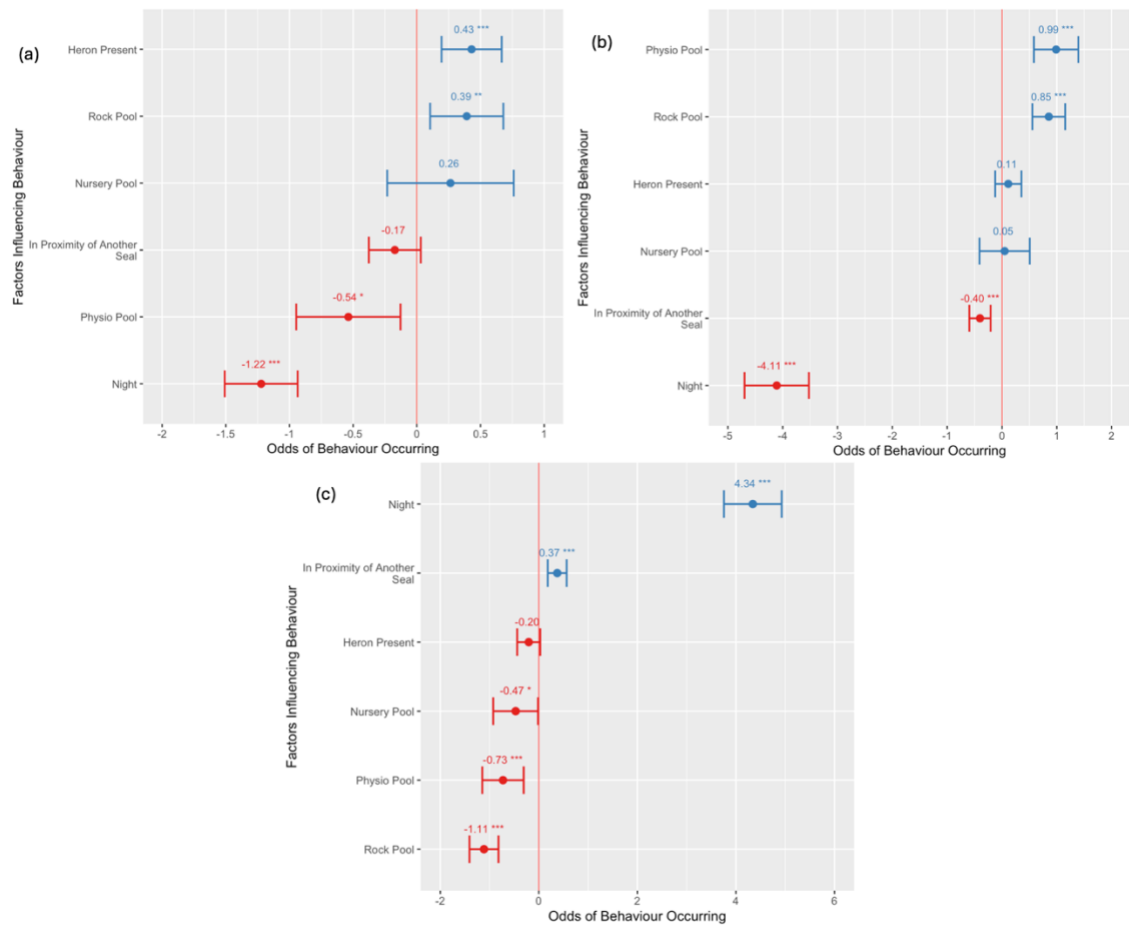
Figure 4: The likelihood of different management practices influencing three grey seal (*Halichoerus grpyus*) behaviours ((a) head elevated, (b) movement, (c) rest) within a rehabilitation centre based in Ireland. Individual ID was factored in as a random effect (N = 31, n = 415). Reference level: Pre-Release Pool, Day, heron absent, and not in proximity of another seal. Blue coloured factors have positive odds of the behaviour occurring and red coloured factors have negative odds of the behaviour occurring. The red line indicates zero and if the factor crosses this line, this factor for this behaviour is more down to chance. P-values are indicated as Asterix's, three Asterix's is <0.001.

4. Discussion:

Investigating the management practices at S.R.I has identified that factors inside the centres control, particularly pool movement and pool type, did not influence weight gain between days but did appear to affect mortality of grey seal pups. Most variables affecting weight gain and mortality were outside of the rehabilitation centres control (e.g., age, pupping season, intake weight, and admittance reason). There were some factors inside the centres control, such as the type of pool, that influenced the behaviour of the grey seal pups. Rock Pool (a large pool with no fencing) was found to have increased likelihood of observing head elevated behaviour and decreased likelihood of observing resting behaviour, when compared with Pre-Release Pool (shielded pool), further suggesting that this pool experiences disturbance from the high footfall area next to the pool.

4.1: Impact of Management Practices:

Previous research on known stressors such as transportation (Kannen et al., 2000; Schuamann et al., 2014), handling (Harris et al., 1998), changes in social grouping (Waples and Gales, 2002; McLaren et al., 2023), and disturbance (Jayakody et al., 2008), affect weight gain. As part of routine practice within rehabilitation centres, seals are moved between pools, which could potentially be causing a detrimental effect on weight gain. However, no evidence was found within this study that routine movement between pools during rehabilitation affected weight gain. This is surprising as it is expected that the individuals would lose weight due to the stress associated with movement. Additionally, as there was no effect of individuals on weight gain, this means that management practices can continue without needing to account for any specific individual.

This study is the first to find evidence that moving individuals between pools, a form of physical disturbance, is associated with mortality. One explanation could be that movement between pools is an acute stressor, as it includes three known stressors (handling, transportation, and changes in social grouping). Sudden disturbances (e.g., sudden movements, abrupt capturing and handling of individual, staff in enclosure at unusual times and numbers) have been shown to increase vigilance behaviour in wild grey seals (Gaspari, 1994), so it is likely that this added stress could be impacting their growth and potentially increasing the risk of instantaneous mortality. A further potential risk is the reduced development of diving physiology. Within marine mammals, there is evidence that physical disturbance has detrimental effects in the development of diving physiology (Noren, 2020), potentially impacting their survival upon release. Although there is no published data on the effect of stress during transportation in seals, transportation is known to be highly stressful for cetaceans as it can cause biochemical conditions that can be fatal (British Small Animal Veterinary Association (BSAVA), 2017). This would suggest that transportation could also be a highly stressful event for seals. Another explanation could be the increased stress from exposing the individual to changes in the social grouping. Changes within the social grouping has been shown to cause stress within social species, such as bottlenose dolphins, with potential for causing weight loss in captive individuals (Waples and Gales, 2002). Furthermore, the pool with the highest behavioural indicators of disturbance was included in the analysis of mortality (to account for the high disturbance indicated within the pool) as it may have explained some of the behavioural responses. This pool (Rock Pool) did not have an effect on mortality and weight gain. This finding was unexpected, but it does not mean that the moves and the pool are not impacting their overall welfare.

While weight gain was not affected, there were observed effects on behaviour. As all three pools are unshielded (Pre-Release Pool is fenced in due to motivation for reducing habituation), visual disturbances are of more concern, as there has been no evidence of habituation occurring within pinnipeds, with most studies unable to link behaviour to habituation (Taylor et al., 1998; Anderson et al., 2011; Paterson et al., 2019). Of the three pools, Rock Pool was found to be the least likely to observe resting behaviour and the most likely pool to observe head elevated behaviour when compared to Pre-Release Pool (shielded pool), suggesting that this could be a high stress pool. However, this result could be explained by the disturbance caused by the high footfall area that is close to this pool. This disturbance could be causing disruption to rest, causing fragmented sleep. Fragmented sleep, in humans, has been associated with impaired alertness, memory, mood regulation, changes to brain activity and metabolism (Short and Banks, 2013). Within harbour seals, there is evidence that disturbance from pedestrians, within 50m distance, impacts resting behaviour when hauled-out (the act of coming onto a solid surface (e.g., land or ice)) on land, as when disturbed, they haul-in (the act of returning into the water after hauling out; can be a very rapid response to fear) reducing the time spent resting and increasing the energetic output (Osinga et al., 2012). This fits with the increased likelihood in observing movement behaviour within this pool and Physio Pool. Fragmented sleep could also explain the increased likelihood of observing head elevated behaviour within Rock Pool. If the high footfall area was causing disturbance within Rock Pool, Physio Pool would have shown similar results as it is also adjacent to the high footfall area. The seal pups are also disturbed during the day for other routine management practices; rehabilitation staff enter the pools to clean or capture individuals for weighing, medication administration or for movement (release or to another pool). Additional evidence was found for when staff enter the pool for cleaning,

there was an increase in likelihood of observing head elevated behaviour. This could also be because they are exposed to a repeated stressor. In meerkats, there is some evidence that an increase in vigilance may occur due to previously experienced stressors through an increase in glucocorticoids (Voellmy et al., 2014).

Physio Pool was found to be the pool with the lowest behavioural indicators of disturbance as it had the lowest likelihood of observing head elevated behaviour and the highest likelihood of observing movement behaviour when compared to Pre-Release Pool. However, during the night, Physio Pool had the highest likelihood of observing head elevated behaviour and during the time period 05:00 – 07:00. This pool is located near the entrance, so could be explained by staff entering the rehabilitation centre as this is the time period in which their volunteers start. Overall, this suggests that this is low disturbance pool and seals are able to exhibit behaviours that are required for their success, e.g., resting, movement, learning to hunt.

The 2022 – 2023 pupping season observed increased likelihood of resting behaviour whilst in proximity of another seal. One explanation for this is that grey seals are known to be social at haul-out sites during the mating/pupping season and moulting season as they congregate in large groups on land. This finding was consistent with Twiss et al., (2022) who also found that grey seals show pro-social behaviours. The increased resting behaviour whilst in proximity of another seal is consistent with behaviour already observed in other seal species (Chilvers et al., 1999), although there is currently no previous evidence of this in grey seals. In harbour seals, evidence has shown that when in groups there is increased allocation of time spent resting in larger and tighter groups (Krieber and Barrette, 1984), which could potentially be reducing stress within the grey seals observed in this study. Additional evidence from Twiss et al., (2022) has shown

that grey seal pups can recognise individuals they have previously encountered, and reduce the level of costly interactions, such as aggression, with these familiars. Therefore, it is necessary for the rehabilitation centre to consider the groupings and/or regroupings as best as possible to avoid certain interactions between individuals, but also the size of the group (this will vary between centres and the number of pups admitted in the year).

A limitation to the behavioural data is that due to camera malfunctions and use of point sampling, it is estimated only 5% of the recorded day footage was collected. Data was not collected between 07:00 and 12:00, so there could be unknown events occurring during this time that could be influencing the behaviours later on, e.g., new individual added into or removed from the group, changing the social dynamic and grouping. However, as data was collected across the day and night, it removes the limitation of events occurring during the night impacting those of the day and *vice versa*. There were expected variations between the night and day data due to more management practices occurring during the day (e.g., pool cleans, weighing, medication), and these effects emerged (e.g., more active during the day, more at rest at night), further validating the approach within this study at capturing ecologically relevant effects. Aggression was not analysed for two reasons; 1) point sampling was not appropriate for this behaviour due to the rare, short-lived behaviours, and 2) the time-lapse camera took a snapshot every 30s which is inadequate for the unpredictability and speed of this behaviour. Aggression would require focal follows and, if to be recorded via time-lapse cameras, would require more frequent timing, e.g., every 10s.

4.2: Impact of Management Practices Outside the Rehabilitation Centre's Control:

There are many factors outside the control of the centre, including the reason for admittance (e.g., disease, injury, and being underweight), age, sex, duration of time within rehabilitation, the initial rate of weight gain, and the pupping season. The main management objective within rehabilitation centres is to maximise weight gain but also to minimise the time in captivity, reducing the risk of habituation, and to minimise the resources required per seal. This study found evidence that disease status, having an injury and being underweight upon admittance had an effect on weight gain, with disease status having a negative effect. Another important finding was that the duration of rehabilitation negatively affects weight gain (i.e., the longer the duration of rehabilitation, the less weight gained between days). Within marine mammals, there has been a lack of research to confirm if the duration of rehabilitation has been related to survival (Wells et al., 2012). This study appears to be the first to find evidence that the total duration of rehabilitation had no effect on mortality. This suggests that although the longer rehabilitation time sees reduced weight gain, it does not cause mortality in the long-term. This could mean that individuals lose weight between days due to stress or because of the initial condition upon admittance. Another finding is that non-dependent individuals (4+ weeks) have a higher probability of mortality than dependents. In wild grey seal pups, mortality is high during the first three months (Hall et al., 2002), with pups under 21-days at particular risk (Harding et al., 2007). One explanation for this could be that within this particular year (2022 – 2023 pupping season), non-dependent individuals were more susceptible to diseases or injury. However, only one deceased individual from this pupping season was admitted with an injury, but this individual was of non-dependent age. A limitation to this model is the

small sample size (N deceased individuals = 6, N alive individuals = 65), so further research is needed to truly understand these factors.

This study confirms that being underweight (84%), having an illness (including parasites (e.g., lungworm or roundworm) and suspected viruses (e.g., seal pox or phocine distemper) (22%), and injury (including entanglement) (52%) are common admittance reasons for grey seals. Furthermore, 73% of mortalities were admitted as underweight. This finding is consistent with Zatrak et al. (2020) where 35% of seals were underweight. Additionally, both this study and Zatrek et al., (2020) had similar classifications for admittance reasons, further providing additional evidence to the concern that underweight individuals are more at risk of entering rehabilitation centres. This high admittance has been previously attributed to interruptions in suckling regime of mother and juveniles (Anderson et al., 1979) as well as post-weaning fasting caused by the learning period (Zatrak et al., 2020). However, Zatrak et al., (2020) sample size was 15.75 times larger than this study (N individuals = 292), and over a 32-year period (1996 – 2020). This larger sample allows for better evaluation of admittance trends. However, since 2020, within this study alone, there has been an increase of individuals admitted with underweight as the cause (180 individuals between 2020 and 2024). This would suggest that underweight individuals are on the rise, which is course for further concern as previous evidence has shown that heavier individuals had higher survival probabilities (Hall et al., 2002, 2008; Zartak et al., 2020). Further evidence from this study revealed that individuals with the admittance reason illness were found to have lower weight gain. This potentially suggests that closer monitoring of these specific individuals is needed. Only within one pupping season (2022 – 2023), seals admitted with an injury had a higher weight gain between days than those without an injury. However, some of the variation within this year was explained by other factors, such as pool changes and

age. Additionally, this study found evidence that the actual weight (kgs) of an individual had a positive correlation with weight gain between days. This adds to the evidence presented by Zatrak et al., (2022); odds of juvenile seal survival increased by 1.05 times per kilogram when over the age predicted weight. There were also significant year effects on weight gain between days, with three years having greater weight gain (2019 -2020, 2020 – 2021, 2022 – 2023). An explanation for this could be that for these years, the sample sizes were larger, potentially capturing more of the variation within these years.

This study found no known factors that had an effect on mortality. Another finding was that there was no effect of initial rate of weight gain (in the first 40-day of rehabilitation) on mortality. The average death occurred at 40 days, but there was a much wider range (minimum: 13 days, maximum: 253 days), potentially meaning that the 40-day threshold is not large enough to capture any changes within weight gain for individuals who stayed for longer and died. Only within one pupping season (2022 – 2023), did this study find evidence that non-dependent individuals (4+ weeks) had a higher probability of mortality. This result is contrary to Zatrak et al. (2020), as 81% of seals within this study were older than four weeks (N individuals = 59) compared with 68.2%. This could explain the higher probability of mortality within non-dependent individuals. However, the sample sizes within Zatrak et al., (2022) were 68.7 times larger than within this study (N individuals = 72), so this study may have lost small changes and trends. However, there were additional factors controlled for in this study, which explained some of the variation; the number of pool moves. A further explanation could be that due to grey seals becoming more solitary as they age (Surviliene et al., 2016), the change in social grouping caused by the pool movements could be causing further stress, that could be cumulative with age. In captive bottlenose dolphins, changes in social grouping has been

shown to cause stress (Waples and Gales, 2002). This could potentially be a very important factor that the rehabilitation centres need to consider when rehabilitating individuals.

Within this study, evidence was found that the likelihood of observing head elevated behaviour increased when herons were present. Herons were included within the combined data as they are one removed from the actual stimulus, "staff feed", as these only occur during the day. Interestingly, within this study there was an unusual presence of herons observed across the pools (20%), with seals observed chasing herons within the pilot data collection. Therefore, it remains unclear within this study if the presence of herons was associated with feeds occurring by the seals or if this a unique interaction between herons and seals. Pinnipeds are known to associate humans with food, as previous evidence has found that fur seals (*Arctocephalus pusillus*) associate fishing vessels and nets with feeding (Shaughnessy et al., 1981). So, it is not unlikely that they could associate herons with the presence of food. Additionally, it could potentially suggest that herons could be used by seals as a use of predictable anthropogenic food sources. Another explanation for the increased likelihood could be that the herons cause a sudden disturbance to the seals, and sudden disturbances have been shown to increase vigilance behaviour in wild grey seals (Gaspari, 1994).

4.3: Individuals within Rehabilitation:

Individuals are a vital part of conserving species, and it is important to make sure that management practices do not impact individuals (Aitken, 2004). Repeatability measures are a way to measure this, allowing for important management practices and decisions to correctly be put in place. In this study, as the repeatability score was low ($R_M$ = <0.2) and statistically significant (p-value = <0.001) (except head elevated behaviour), current

management practices in place are not having an effect at the individual level. As all individuals within this study were pups and not adults, it is possible that the behavioural traits have not developed into behavioural syndromes yet. A 2015 review reported evidence that in juveniles, behavioural repeatability is often lower than adults (Brommer and Class, 2015). Brommer and Class (2015) theorised that the changes in behavioural repeatability and consistency across the life history do not contradict behavioural syndrome, further emphasising that it is important to investigate personality traits across the different life history stages. These results mean that management practices such as the level of water within a pool, do not affect one individual more than another individual, removing the need to cater for particular individuals within a management decision.

4.3: Recommendations and Further Research:

It would be advisable to monitor the practice of moving individuals frequently between pools, as further research is required to fully unpick this due to the small sample size (N individuals = 31). This study assumes that pool moves causes stress hence mortality, but there could be systematic reasons individuals are moved which may potentially cause mortality directly (e.g., removal of individual due to injury, increased severity of condition, at risk of deterioration due to social context). Additionally, it would be advisable to monitor the weight of diseased individuals more closely due to the lower weight gain between days. Additionally, a recommendation from this study to the S.R.I. is to monitor the Rock Pool and try to implement some form of mediation to reduce the potential stress experienced within this pool, such as reducing the fragmented sleep. This could include putting a small fence that is opaque to reduce the exposure of seals to the high footfall area. It is also advisable that pool designs should now include a fence,

creating a visual barrier between seals and humans, further removing the effect of disturbance from the presence of people.

I could not summarise the data in a reasonable way per individual in order to compare behaviour and weight change. As the behavioural data was collected through point sampling over shorter time periods (snapshot every 30s), and the weight data collected over longer time periods (once a week), it was not possible to derive one summary measure from varied numbers of point samples across the different conditions. Due to this inability, it remains unknown if the pool with the highest indicator of disturbances or the number of moves did cause any weight changes. Due to the S.R.I.'s additional software malfunctions and data loss, it was difficult to investigate pool moves over more than one year, so further research is required in this area to fully understand the effects of the number of moves between pools on weight and mortality, as the sample size was small. Additionally, it is possible that the threshold set for initial weight gain from intake (40 days from intake), is not related to the cause of death. Additional research on the interactions between environmental cues and seals to feeding would further help with understanding the relationship to head elevated behaviour, and if this is vigilance behaviour or if it is more specifically related to the presence food. Additionally, stress can evoke a change in the exploratory response with some individuals becoming more exploitive of resources when on an unpredictable cycle (Matisz et al., 2021). This creates a further problem as although it is ideal to vary the times of feeding to reduce the association of feed with environmental cues, it might alter the behavioural responses of individuals. Based upon this, it would be advisable to follow a predictable food cycle as for seal pups, associating herons with food may give them extra opportunities to forage upon release.

5. Conclusion:

While there are many known rehabilitation management practices that are outside the control of the centre that are influencing weight gain, mortality, and behaviour, this study has found that there are unknown management practices within the control of the centre that are influencing the mortality of individuals, i.e., pool movement. Therefore, I recommend that the rehabilitation centre pay particular attention to the number of pool moves. Finally, there is a need for an alternate record keeping system to allow for the best utilisation of data as this study was only able to look at pool movement within one seal pupping season due to the malfunction within the current record keeping system. The alternate record keeping system would further allow research into pool movements across years, further optimising seal rehabilitation protocols in the future. It would also give scope to inform management as well as fundamentally understand wild populations.

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**Appendix:**

**A.1: Behavioural Statistical Methods:**

Behaviour was analysed using generalised mixed effects models (GLMM's) on Night (n = 90 observations, N = 27 seals), and Day (n = 334 observations, N = 30 seals), using package "lme4" (Bates et al., 2015), for three behaviours (head elevated, movement, and rest). Aggression was not analysed due to rarity of behaviour (Night: n = 2 observations; Day: n = 28 observations).

Fixed independents variables for each dataset (See Table A1 for reference levels); Day: pool, hour, water level, staff feed, staff intensity, and in proximity of another seal; Night: pool, time period, weather, and in proximity of another seal. Individual ID was included in each analysis as a random effect as it was determined to be an important variable through comparing AIC numbers (see Supplementary 4 for further details). All unidentifiable individuals were removed from the analysis. Confounding effects were tested for using package "car" (Fox and Weisberg, 2019).

Table A1: Reference levels for fixed independent variables for behavioural generalised linear mixed effects models (GLMER's).

| Fixed Independent Variable | Reference Level | Data Analysis Used In |
|---|---|---|
| Pool | Pre-release Pool. | Both. |
| In Proximity of Another Seal | Not in proximity of another seal. | Both. |
| Hour | 12:00 – 13:00. | Day. |
| Water Level | No water in the pool (0). | Day. |
| Staff feed | No staff feed occurring. | Day. |
| Staff intensity | No staff intensity (level 0). | Day. |
| Time Period | 00:00 – 01:00 (Time period 1). | Night. |
| Weather | Dry | Night. |

Consistency repeatability estimates, following Nakagawa and Schielzeth, 2010, determined if the behaviours were behavioural syndromes, using package "rptR" (Stoffel et al., 2017). Estimated adjusted repeatability (an estimate adjusting for confounding effects) was used as there were confounding effects within the GLMM's. To be classed as a behavioural syndrome, the estimates needed to be higher than 0.2 (Harper 1994).

See results in Supplementary 5.

**A.2: Justification for removing certain admittance reasons from the model.**

Table A2: Justification for excluding admittance reasons from analyses:

| Admittance Reasons | Reasons for exclusion |
|---|---|
| Premature | Rarity of occurrence (n = 29) and potential inconsistent reporting. |
| Harassed | Rarity of occurrence (n = 40) and potential inconsistent reporting. |
| Dehydrated | Correlated with injured (Pearson's chi-square test: X-squared = 0.22, p-value = 0.64) and illness (Pearson's chi-square test: X-squared = 0, p-value = 1). |
| Emaciated | Combined with underweight due to and potential inconsistent reporting. |
| Bad location | Rarity of occurrence (n = 19), and colinear with age (Pearson's chi-square test: X-squared = 2.57, df = 2, p-value = 0.2767). |
| Entanglement | Combined with injured due to close similarity in definitions. |
| Orphaned | Potential inconsistent reporting: individuals in non-dependent age class (already abandoned) have this admittance reason (n = 3). |

**Supplementary Information:**

**S.1: Definitions of Reason for Admittance:**

Table S1: Definitions of the Different Admittance Reasons for Grey Seals (*Halichoerus grypus*) Admitted to Seal Rescue Ireland, Ireland. These definitions have been provided through personal communication with Seal Rescue Ireland. These can vary slightly between people admitting the seal.

| Admittance Reason | Definition |
|---|---|
| Emaciated | Seal is extremely underweight for their age, with very visible bones and rolls of skin. Young pups severely under normal birth weight around 15kgs are also classed as emaciated. |
| Underweight | Seal is underweight, has some visible bones and rolls of skin, closer to their birth weight for young pups. If weaned, greys coming in around 18-20kg are underweight. It depends on the body condition relative to the seal's size (length) and rough age. |
| Illness | Seals with evident parasites e.g., lungworms, roundworms or if the seal has alopecia, or with virus e.g., phocine distemper virus, seal pox |
| Injured | This ranges from small scratches/grazes anywhere on the body to large open wounds. |
| Dehydrated | Seals who have no eye rings and very small-looking eyes. |
| Orphaned | Seals who are dependent on their mother's milk to survive and have been separated prematurely before being weaned. |
| Premature | Grey seals born small and without teeth are classed as premature. |
| Entanglement | Seals with active entanglement or evidence that there was an ex-entanglement (scarring). |
| Bad Location | Seal that seems relatively healthy and can survive without rehab will need to be relocated, for example seal is on a busy beach and being harassed, or the seal has found itself far from the sea etc. |
| Harassed | Seals healthy, injured, sick or orphaned that are approached continually by members of the public, lifted by members of the public etc, not allowing the seal to rest. |
| Unknown | Admittance reason has not been recorded or it does not fall into one of the above categories. |

## S.2: Camera locations



Figure S1 (left): Reolink Go PT Plus (Reolink, China) in Nursery pool (camera 2). Camera is attached to the pole using supplied strap. Solar panel charges the camera and is placed in a place that will get direct sunlight to be able to charge the cameras.

Figure S2 (Right): Reolink Go PT Plus (Reolink, China) in Nursery pool (camera 3). Camera is attached to the pole using supplied strap. Solar panel charges the camera and is placed in a place that will get direct sunlight to be able to charge the cameras.
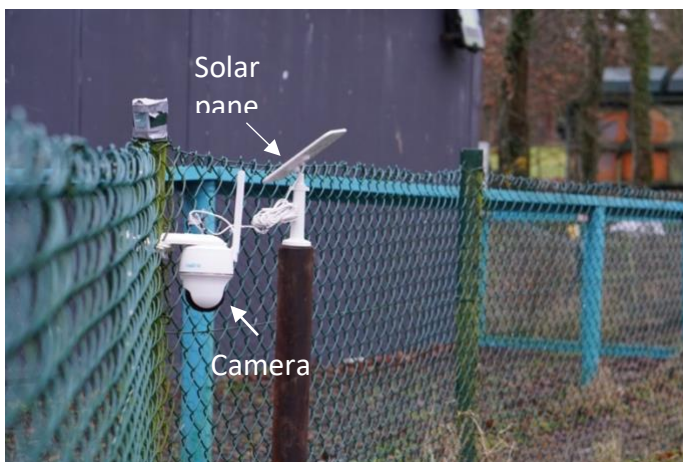




Figure S3 (left): Reolink Go PT Plus (Reolink, China) in Nursery pool (camera 4). Camera is attached to the pole using supplied strap. Solar panel charges the camera and is placed in a place that will get direct sunlight to be able to charge the cameras.

## S.3: AIC Numbers for Comparison of Behavioural Models

An anova test was used to compare the two models and look at the importance of the random effect within the models: GLM and GLMER. The random effect in the GLMER model was Field ID.

### S.3.1: Combined Behavioural Dataset:

Table S3.1.1: Anova Test for AIC Numbers for Comparison of GLM to GLMER Models for Movement Behaviour:

| Model | npar | AIC | BIC | logLik | Deviance | Chisq | DF | Pr(>Chisq) |
|-------|------|-----|-----|--------|----------|-------|----|-----------|
| GLM | 7 | 1306.1 | 1334.3 | -646.04 | 1292.1 | | | |
| GLMER | 8 | 1263.5 | 1295.8 | -623.77 | 1274.5 | 44.534 | 1 | 2.5e-11 |

Table S3.1.2: Anova Test for AIC Numbers for Comparison of GLM to GLMER Models for Resting Behaviour:

| Model | npar | AIC | BIC | logLik | Deviance | Chisq | DF | Pr(>Chisq) |
|-------|------|-----|-----|--------|----------|-------|----|-----------|
| GLM | 7 | 1343.8 | 1372 | -664.91 | 1329.8 | | | |
| GLMER | 8 | 1286.7 | 1318.9 | -635.35 | 1270.7 | 59.13 | 1 | 1.476e-14 |

Table S3.1.3: Anova Test for AIC Numbers for Comparison of GLM to GLMER Models for Head Elevated Behaviour:

| Model | npar | AIC | BIC | logLik | Deviance | Chisq | DF | Pr(>Chisq) |
|-------|------|-----|-----|--------|----------|-------|----|-----------|
| GLM | 7 | 1259.6 | 1287.8 | -622.80 | 1245.6 | | | |
| GLMER | 8 | 1247.9 | 1280.1 | -615.94 | 1231.9 | 13.719 | 1 | 0.0002123 |

### S.3.2: Day Behavioural Dataset:

Table S3.2.1: Anova Test for AIC Numbers for Comparison of GLM to GLMER Models for Movement behaviour:

| Model | npar | AIC | BIC | logLik | Deviance | Chisq | DF | Pr(>Chisq) |
|-------|------|-----|-----|--------|----------|-------|----|-----------|
| GLM | 14 | 1220.0 | 1273.4 | -596.01 | 1192.0 | | | |
| GLMER | 15 | 1178.6 | 1235.7 | -574.28 | 1148.6 | 43.455 | 1 | 4.338e-11 |

Table S3.2.2: Anova Test for AIC Numbers for Comparison of GLM to GLMER Models for Resting Behaviour:

| Model | npar | AIC | BIC | logLik | Deviance | Chisq | DF | Pr(>Chisq) |
|-------|------|-----|-----|--------|----------|-------|----|-----------|
| GLM | 14 | 1283.9 | 1337.2 | -627.95 | 1255.9 | | | |
| GLMER | 15 | 1229.9 | 1287.1 | -599.96 | 1199.9 | 55.97 | 1 | 7.358e-14 |

Table S3.2.3: Anova Test for AIC Numbers for Comparison of GLM to GLMER Models for Head Elevated Behaviour:

| Model | npar | AIC | BIC | logLik | Deviance | Chisq | DF | Pr(>Chisq) |
|-------|------|-----|-----|--------|----------|-------|----|-----------|
| GLM | 14 | 1021.6 | 1074.9 | -496.79 | 993.57 | | | |
| GLMER | 15 | 1012.9 | 1070.1 | -491.44 | 982.89 | 10.683 | 1 | 0.001081 |

S.3.3: Night Behavioural Dataset:

Table S3.3.1: Anova Test for AIC Numbers for Comparison of GLM to GLMER Models for Movement behaviour:

| Model | npar | AIC | BIC | logLik | Deviance | Chisq | DF | Pr(>Chisq) |
|---|---|---|---|---|---|---|---|---|
| GLM | 8 | 296.69 | 316.68 | -140.34 | 280.69 | | | |
| GLMER | 9 | 269.50 | 292.00 | -125.75 | 251.50 | 29.184 | 1 | 6.582e-08 |

Table S3.3.2: Anova Test for AIC Numbers for Comparison of GLM to GLMER Models for Resting Behaviour:

| Model | npar | AIC | BIC | logLik | Deviance | Chisq | DF | Pr(>Chisq) |
|---|---|---|---|---|---|---|---|---|
| GLM | 6 | 209.01 | 224.01 | -98.505 | 197.01 | | | |
| GLMER | 7 | 193.84 | 211.34 | -89.920 | 179.84 | 17.17 | 1 | 3.418e-05 |

Table S3.3.3: Anova Test for AIC Numbers for Comparison of GLM to GLMER Models for Head Elevated Behaviour.

| Model | npar | AIC | BIC | logLik | Deviance | Chisq | DF | Pr(>Chisq) |
|---|---|---|---|---|---|---|---|---|
| GLM | 8 | 661.10 | 681.10 | -322.55 | 645.10 | | | |
| GLMER | 9 | 352.16 | 374.66 | -167.08 | 334.16 | 310.95 | 1 | < 2.2e-16 |

# S.4: Behaviour Model Results (Including Repeatability).

## S4.1: Day Data:

S4.1.1: Movement Behaviour:

Table S4: Variables Influencing the likelihood of observing movement behaviour in pupping year 2022-2023 (n = 30 seals, N = 334 observations). Reference level: Pre-Release Pool, 12:00 – 13:00, little to no water present in the pool, no staff feeds, no intensity of staff in the pool, and not in proximity of another seal. Random effects are individual ID, residual variance ($\sigma^2$) = 3.29, Between subject variance ($\tau_{00}$) = 0.18, ICC = 0.05, Marginal $R^2$ = 0.094, Conditional $R^2$ = 0.140.

| Predictors | Estimate | Confidence interval | p-value |
|---|---|---|---|
| (Intercept) | 0.26 | 0.14 – 0.50 | **<0.001** |
| Nursery Pool | 0.85 | 0.52 – 1.38 | 0.509 |
| Rock Pool | 1.67 | 1.15 – 2.41 | **0.006** |
| Physio Pool | 2.22 | 1.44 – 3.42 | **<0.001** |
| 13:00 – 14:00 | 1.38 | 1.01 – 1.88 | **0.044** |
| 14:00 – 15:00 | 1.52 | 1.11 – 2.06 | **0.008** |
| 15:00 – 16:00 | 1.57 | 1.15 – 2.15 | **0.004** |
| Shoulder Depth Water Level | 0.89 | 0.52 – 1.53 | 0.673 |
| Swimmable Water Level | 1.66 | 0.98 – 2.79 | 0.059 |
| Staff Feed (Yes) | 1.22 | 0.72 – 2.07 | 0.454 |
| Low Intensity of Staff | 1.28 | 0.82 – 2.01 | 0.276 |
| Medium Intensity of Staff | 0.63 | 0.36 – 1.09 | 0.099 |
| High Intensity of Staff | 1.87 | 0.89 – 3.96 | 0.100 |
| In Proximity of Another Seal | 0.65 | 0.53 – 0.81 | **<0.001** |

Table S5: Link-Scale Approximation and Original-Scale Approximation for Repeatability of Movement Behaviour.

*Link-Scale Approximation*

| | |
|---|---|
| R | 0.024 |
| Standard Error | 0.012 |
| Confidence Interval | [0, 0.047] |
| p-value | 0.000926 [LRT] |
| | NA [Permutation] |

S4.1.2: Rest Behaviour:

Table S6: Variables influencing the likelihood of observing resting behaviour in pupping year 2022-2023 (n = 30 seals, N = 334 observations). Reference level: Pre-Release Pool, 12:00 – 13:00, little to no water present in the pool, no staff feeds, no intensity of staff in the pool, and not in proximity of another seal. Random effects are individual ID, residual variance ($\sigma^2$) = 3.29, Between subject variance ($\tau_{00}$) = 0.22, ICC = 0.06, Marginal $R^2$ = 0.082, Conditional $R^2$ = 0.139.

| Predictors | Estimates | Confidence Interval | p-value |
|---|---|---|---|
| (Intercept) | 2.28 | 1.24 – 4.20 | 0.008 |
| Nursery Pool | 0.70 | 0.43 – 1.14 | 0.152 |
| Rock Pool | 0.34 | 0.23 – 0.49 | <0.001 |
| Physio Pool | 0.52 | 0.33 – 0.82 | 0.005 |
| 13:00 – 14:00 | 0.72 | 0.53 – 0.96 | 0.028 |
| 14:00 – 15:00 | 0.71 | 0.53 – 0.95 | 0.022 |
| 15:00 – 16:00 | 0.53 | 0.39 – 0.72 | <0.001 |
| Shoulder Depth Water Level | 1.04 | 0.63 – 1.72 | 0.868 |
| Swimmable Water Level | 0.96 | 0.60 – 1.56 | 0.879 |
| Staff Feed (Yes) | 0.88 | 0.52 – 1.50 | 0.647 |
| Low Intensity of Staff | 1.14 | 0.73 – 1.77 | 0.577 |
| Medium Intensity of Staff | 1.63 | 0.99 – 2.69 | 0.056 |
| High Intensity of Staff | 0.93 | 0.45 – 1.91 | 0.844 |
| In Proximity of Another Seal | 1.51 | 1.22 – 1.86 | <0.001 |

Table S7: Link-Scale Approximation and Original-Scale Approximation for Repeatability of Rest Behaviour.

Link-Scale Approximation

| | |
|---|---|
| R | 0.031 |
| Standard Error | 0.014 |
| Confidence Interval | [0.002, 0.059] |
| p-value | 0.000236 [LRT] |
| | NA [Permutation] |

S4.1.3: Head Elevated Behaviour:

Table S8: Variables influencing the likelihood of observing head elevated behaviour in pupping year 2022-2023 (n = 30 seals, N = 334 observations). Reference level: Pre-Release Pool, 12:00 – 13:00, little to no water present in the pool, no staff feeds, no intensity of staff in the pool, and not in proximity of another seal. Random effects are individual ID, residual variance ($\sigma^2$) = 3.29, Between subject variance ($\tau_{00}$) = 0.12, ICC = 0.03, Marginal $R^2$ = 0.079, Conditional $R^2$ = 0.111.

| Predictors | Estimates | Confidence Interval | p-value |
|---|---|---|---|
| (Intercept) | 0.45 | 0.23 – 0.89 | 0.022 |
| Nursery Pool | 0.65 | 0.38 – 1.12 | 0.118 |

| | | | |
|---|---|---|---|
| Rock Pool | 1.62 | 1.11 – 2.38 | **0.013** |
| Physio Pool | 0.53 | 0.34 – 0.82 | **0.005** |
| 13:00 – 14:00 | 0.94 | 0.66 – 1.33 | 0.711 |
| 14:00 – 15:00 | 0.82 | 0.58 – 1.16 | 0.264 |
| 15:00 – 16:00 | 1.45 | 1.03 – 2.06 | **0.033** |
| Shoulder Depth Water Level | 0.96 | 0.55 – 1.68 | 0.881 |
| Swimmable Water Level | 0.62 | 0.35 – 1.08 | 0.090 |
| Staff Feed (Yes) | 0.99 | 0.55 – 1.80 | 0.984 |
| Low Intensity of Staff | 1.10 | 0.68 – 1.76 | 0.702 |
| Medium Intensity of Staff | 2.13 | 1.23 – 3.66 | **0.007** |
| High Intensity of Staff | 0.52 | 0.23 – 1.19 | 0.122 |
| In Proximity of Another Seal | 0.93 | 0.73 – 1.18 | 0.560 |

Table S9: Link-Scale Approximation and Original-Scale Approximation for Repeatability of Alertness (Head Up) Behaviour.

*Link-Scale Approximation*

| | |
|---|---|
| R | 0.012 |
| Standard Error | 0.008 |
| Confidence Interval | [0, 0.027] |
| p-value | 0.0741 [LRT] |
| | NA [Permutation] |

S4.2: Night Data:

S4.2.1: Movement Behaviour:

Table S10: Variables influencing the likelihood of observing movement behaviour in pupping year 2022-2023 (n = 26 seals, N = 90 observations). Reference level: Pre-Release Pool, 00:00 – 01:00, dry, and not in proximity of another seal. Random effects are individual ID, residual variance ($\sigma^2$) = 3.29, Between subject variance ($\tau_{00}$) = 1.60, ICC = 0.33, Marginal $R^2$ = 0.121, Conditional $R^2$ = 0.408.

| Predictors | Estimates | Confidence Interval | p-value |
|---|---|---|---|
| (Intercept) | 0.03 | 0.01 – 0.10 | **<0.001** |
| Nursery Pool | 1.03 | 0.21 – 5.03 | 0.975 |
| Rock Pool | 0.67 | 0.17 – 2.58 | 0.561 |
| Physio Pool | 0.25 | 0.06 – 1.08 | 0.063 |
| 02:00 – 04:00 | 0.40 | 0.07 – 2.27 | 0.298 |
| 05:00 – 07:00 | 1.17 | 0.60 – 2.25 | 0.647 |
| Rain | 0.75 | 0.27 – 2.10 | 0.581 |
| In Proximity of Another Seal | 0.56 | 0.23 – 1.35 | 0.195 |

Table S11: Link-Scale Approximation and Original-Scale Approximation for
Repeatability of Movement Behaviour.

*Link-Scale Approximation*

| | |
|---|---|
| *R* | 0 |
| *Standard Error* | 0.005 |
| *Confidence Interval* | [0, 0.015] |
| *p-value* | 1 [LRT] |
| | NA [Permutation] |

S4.2.2: Rest Behaviour:

Table S12: Variables influencing the likelihood of observing resting behaviour in pupping
year 2022-2023 (n = 26 seals, N = 90 observations). Reference level: Pre-Release Pool,
dry, and not in proximity of another seal. Time period was removed from this analysis
due to insufficient data within time period 02:00 – 04:00 (N = 600). Random effects are
individual ID, residual variance ($\sigma^2$) = 3.29, Between subject variance ($\tau_{00}$) = 2.11, ICC =
0.39, Marginal $R^2$ = 0.176, Conditional $R^2$ = 0.497.

| *Predictors* | *Estimates* | *Confidence Interval* | *p-value* |
|---|---|---|---|
| *(Intercept)* | 42.61 | 10.13 – 179.31 | **<0.001** |
| *Nursery Pool* | 1.62 | 0.17 – 15.72 | 0.680 |
| *Rock Pool* | 6.87 | 1.10 – 43.03 | **0.040** |
| *Physio Pool* | 7.38 | 1.10 – 49.56 | **0.040** |
| *Rain* | 0.74 | 0.19 – 2.83 | 0.661 |
| *In Proximity of Another Seal* | 2.88 | 0.76 – 10.97 | 0.120 |

Table S13: Link-Scale Approximation and Original-Scale Approximation for
Repeatability of Rest Behaviour.

*Link-Scale Approximation*

| | |
|---|---|
| *R* | 0 |
| *Standard Error* | 0.01 |
| *Confidence Interval* | [0, 0.007] |
| *p-value* | 1 [LRT] |
| | NA [Permutation] |

S4.2.3: Head Elevated Behaviour:

Table S14: Variables influencing the likelihood of observing head elevated behaviour in pupping year 2022-2023 (n = 26 seals, N = 90 observations). Reference level: Pre-Release Pool, 00:00 – 01:00, dry, and not in proximity of another seal. Random effects are individual ID, residual variance ($\sigma^2$) = 3.29, Between subject variance ($\tau_{00}$) = 5.26, ICC = 0.62, Marginal $R^2$ = 0.340, Conditional $R^2$ = 0.746.

| Predictors | Estimates | Confidence Interval | p-value |
|---|---|---|---|
| (Intercept) | 0.16 | 0.04 – 0.71 | **0.016** |
| Nursery Pool | 0.93 | 0.17 – 5.11 | 0.932 |
| Rock Pool | 0.21 | 0.04 – 1.06 | 0.059 |
| Physio Pool | 0.03 | 0.00 – 0.25 | **0.001** |
| 02:00 – 04:00 | 0.27 | 0.06 – 1.24 | 0.092 |
| 05:00 – 07:00 | 4.49 | 2.92 – 6.90 | **<0.001** |
| Rain | 1.08 | 0.37 – 3.16 | 0.889 |
| In Proximity of Another Seal | 0.17 | 0.09 – 0.32 | **<0.001** |

Table S15: Link-Scale Approximation and Original-Scale Approximation for Repeatability of Alertness (Head Up) Behaviour.

*Link-Scale Approximation*

| | |
|---|---|
| R | 0.095 |
| Standard Error | 0.058 |
| Confidence Interval | [0, 0.214] |
| p-value | 0.00601 [LRT] |
| | NA [Permutation] |

S4.3: Combined Night and Day

S4.3.1: Movement Behaviour:

Table S16: Variables influencing the likelihood of observing movement behaviour in pupping year 2022-2023 (n = 31 seals, N = 415 observations). Reference level: Pre-Release Pool, day, heron absent, and not in proximity of another seal. Random effects are individual ID. Random effects are individual ID, residual variance ($\sigma^2$) = 3.29, Between subject variance ($\tau_{00}$) = 0.16, ICC = 0.05, Marginal $R^2$ = 0.477, Conditional $R^2$ = 0.502.

| Predictors | Estimate | Confidence interval | p-value |
|---|---|---|---|
| (Intercept) | 0.41 | 0.29 – 0.58 | **<0.001** |
| Nursery Pool | 1.05 | 0.66 – 1.66 | 0.835 |
| Rock Pool | 2.35 | 1.74 – 3.17 | **<0.001** |
| Physio Pool | 2.69 | 1.79 – 4.03 | **<0.001** |
| Night | 0.02 | 0.01 – 0.03 | **<0.001** |
| Heron Present | 1.12 | 0.88 – 1.42 | 0.346 |
| In Proximity of Another Seal | 0.67 | 0.55 – 0.81 | **<0.001** |

Table S17: Link-Scale Approximation and Original-Scale Approximation for Repeatability of Movement Behaviour.

*Link-Scale Approximation*

| | |
|---|---|
| *R* | 0.014 |
| *Standard Error* | 0.008 |
| *Confidence Interval* | [0, 0.028] |
| *p-value* | 0.00094 [LRT] |
| | NA [Permutation] |

S4.3.2: Rest Behaviour:

Table S18: Variables influencing the likelihood of observing resting behaviour in pupping year 2022-2023 (n = 31 seals, N = 415 observations). Reference level: Pre-Release Pool, day, heron absent, and not in proximity of another seal. Random effects are individual ID, residual variance ($\sigma^2$) = 3.29, Between subject variance ($\tau_{00}$) = 0.21, ICC = 0.06, Marginal $R^2$ = 0.508, Conditional $R^2$ = 0.537.

| Predictors | Estimate | Confidence interval | p-value |
|---|---|---|---|
| *(Intercept)* | 1.86 | 1.29 – 2.66 | **0.001** |
| *Nursery Pool* | 0.62 | 0.40 – 0.98 | **0.042** |
| *Rock Pool* | 0.33 | 0.24 – 0.44 | **<0.001** |
| *Physio Pool* | 0.48 | 0.32 – 0.74 | **0.001** |
| *Night* | 76.89 | 42.79 – 138.16 | **<0.001** |
| *Heron Present* | 1.12 | 0.64 – 1.03 | 0.088 |
| *In Proximity of Another Seal* | 1.45 | 1.20 – 1.76 | **<0.001** |

Table S19: Link-Scale Approximation and Original-Scale Approximation for Repeatability of Rest Behaviour.

*Link-Scale Approximation*

| | |
|---|---|
| *R* | 0.017 |
| *Standard Error* | 0.009 |
| *Confidence Interval* | [0, 0.033] |
| *p-value* | 0.000122 [LRT] |
| | NA [Permutation] |

S4.3.3: Head Elevated Behaviour:

Table S20: Variables influencing the likelihood of observing head elevated behaviour in pupping year 2022-2023 (n = 31 seals, N = 415 observations). Reference level: Pre-Release Pool, day, heron absent, and not in proximity of another seal. Random effects are individual ID, residual variance ($\sigma^2$) = 3.29, Between subject variance ($\tau_{00}$) = 0.15, ICC = 0.04, Marginal $R^2$ = 0.135, Conditional $R^2$ = 0.172.

| Predictors | Estimate | Confidence interval | p-value |
|---|---|---|---|
| *(Intercept)* | 0.29 | 0.21 – 0.40 | **<0.001** |

| | | | |
|---|---|---|---|
| Nursery Pool | 1.30 | 0.79 – 2.14 | 0.297 |
| Rock Pool | 1.48 | 1.11 – 1.97 | **0.008** |
| Physio Pool | 0.58 | 0.39 – 0.88 | **0.010** |
| Night | 0.29 | 0.22 – 0.39 | **<0.001** |
| Heron Present | 1.54 | 1.21 – 1.95 | **<0.001** |
| In Proximity of Another Seal | 0.84 | 0.69 – 1.03 | 0.097 |

Table S21: Link-Scale Approximation and Original-Scale Approximation for Repeatability of Alertness (Head Up) Behaviour.

*Link-Scale Approximation*

| | |
|---|---|
| *R* | 0.008 |
| *Standard Error* | 0.005 |
| *Confidence Interval* | [0, 0.018] |
| *p-value* | 0.193 [LRT] |
| | NA [Permutation] |