Modelling energetics of fur seals and sea lions

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Declaration

Except where acknowledged in the customary manner, the material presented in this thesis to the best of my knowledge, original and has not been submitted in whole or part for a degree in any University.



Monique Ladds

Ethics statement

All experiments in this thesis were carried out with Macquarie University ethics committee approval (ARA-2012_064) and Taronga ethics committee (4c/10/13). All experiments were conducted under the current laws of Australia authorised under New South Wales Office of Environment and Heritage Scientific Licence SL100746. All animal handling and experimental procedures in Canada were conducted in accordance with regulations of the Canadian Council on Animal Care (University of British Columbia animal use permit #A11-0397), Department of Fisheries and Oceans Canada (MML 2007-001) and the Vancouver Aquarium.

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Abstract

Energy is the source of life, and at the most basic level, survival of a species is dictated by how efficiently animals gain and use their accumulated energy. Energetics, the study of how animals organise their daily or seasonal activities, allows us to understand how animal energy budgets are used and affected by life history or environmental changes. However, to comprehend fully how animals may respond to changes in their environment it is necessary to measure accurately behaviour and physiology of wild animals. This can be achieved by estimating time-energy budgets from accelerometers. In this thesis, energetic models were developed through experiments with a diverse group of captive fur seals and sea lions (otariids) and subsequently applied to wild fur seals. Otariids are an excellent study species as they predictably haul out at the same colony after foraging at sea (for data recovery) and must return to the surface to breathe (for energetics studies). Captive surrogates were used to develop baseline energetic data for the species of interest: Australian fur seals, New Zealand fur seals and Australian sea lions. The standard metabolic rate of otariids was measured over a year. Females were found to have generally higher metabolic rates than males, and fur seals, but not sea lions, have predictable changes in metabolic rate related to time of year and moult. The metabolic rate of activity was investigated by training adult and subadult otariids to swim submerged for varying durations. Otariids exhibited a dive response, as their metabolic rate decreased with extended periods of swimming, and juveniles had an additional cost of movement. Swimming trials with otariids swimming or diving to feeding tubes were used to validate accelerometers for measuring energy expenditure. Dive duration, total stroke rate and dynamic body acceleration (DBA) was found to accurately predict total energy expenditure for a swim and DBA explained more variation in the model than stroke rate and dive duration. The use of accelerometers to distinguish among groups of four ecologically important behaviours (grooming, foraging, travelling and resting) was validated on captive surrogates and time-energy budgets were developed from this information. These time-energy budgets, when applied to wild fur seals, indicated juvenile fur seals change their behaviour to cope with different energetic pressures between seasons. Juvenile fur seals also have higher energetic costs than adults that may limit their scope for increasing foraging effort during times of resource limitation. These findings indicate that time-energy budgets measured with accelerometers are a useful method of monitoring populations of seals over time that may be subject to limited food availability.

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List of publications

This thesis is formatted in the "thesis by publication" style, where the data chapters are a combination of papers published in international journals and unpublished papers (currently under review) intended for future publication in peer-reviewed scientific journals. Each chapter has specific project aims to address different components of this thesis. They are then drawn together in Chapter 8 to demonstrate the application of the findings. A final conclusion and future direction chapter summarises the thesis.

I am the lead author of all publications listed with the most significant contribution. Macquarie University policy requires that: (1) these papers form a coherent body of work and; (2) the contribution of myself and others is specified.

To satisfy point 1, the thesis is presented in such a way that each chapter builds on the methods and results of the previous chapter, culminating in a final discussion paper that uses the methods and results of all previous chapters. Text, figures and tables have all been formatted with a consistent style for the thesis, and most chapters include additional information that was not included for publication.

List of publications

Chapter 2: Ladds, MA, Slip, DJ and Harcourt, RG (2017). Intrinsic and extrinsic influences on standard metabolic rates of three species of Australian otariid. Conservation Physiology 5(1). DOI: 10.1093/conphys/cow074.

<u>M.L.</u> conceived the study design, collected data, performed data analysis and drafted the manuscript. R.H. and D.S. were involved in study design, data collection and writing.

Chapter 3: Ladds, MA, Slip, DJ and Harcourt, RG (2016). Swimming metabolic rates vary by sex and development stage, but not by species, in three species of Australian otariid seals. Journal of Comparative Physiology B 187(3): 503–516. DOI: 10.1007/s00360-016-1046-5.

<u>M.L.</u> conceived the study design, collected data, performed data analysis and drafted the manuscript. R.H. and D.S. were involved in study design, data collection and writing.

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<u>M.L.</u> conceived the study design, collected data, performed data analysis and drafted the manuscript. A.T. performed data analysis and contributed to writing. D.H., R.H. and D.S. were involved in study design, data collection and writing.

Chapter 5: Ladds, MA, Thompson, AP, Kadar, J-P, J Slip, D, P Hocking, D and G Harcourt, R (2017). Super machine learning: improving accuracy and reducing variance of behaviour classification from accelerometry. Animal Biotelemetry 5(1): 8. DOI: 10.1186/s40317-017-0123-1. <u>M.L.</u> conceived the study design, collected data, performed data analysis and drafted the manuscript. A.T. performed data analysis and contributed to writing. D.H., R.H. and D.S. were involved in study design, data collection and writing. J.K. was involved in data collection, performed data analysis and contributed to writing.

Chapter 6: Ladds, MA, Rosen, DA, Slip, DJ and Harcourt, RG (under review). Validating accelerometers to predict stroke rate using captive fur seals and sea lions. Journal of Experimental Biology.

<u>M.L.</u> conceived the study design, collected data, performed data analysis and drafted the manuscript. D.R., R.H. and D.S. were involved in study design, data collection and writing.

Chapter 7: Ladds, MA, Rosen, DA, Slip, DJ and Harcourt, RG (under review). Proxies of energy expenditure for marine mammals: an experimental study of "the time trap". Frontiers in Aquatic Physiology.

<u>M.L.</u> conceived the study design, collected data, performed data analysis and drafted the manuscript. D.R., R.H. and D.S. were involved in study design, data collection and writing.

Chapter 8: Ladds, MA, Salton, M, Hocking, D, McIntosh, R, Thompson, A, Slip, DJ and Harcourt, RG (under review). Accelerometers recording at low frequencies can be used to develop time-energy budgets of wild fur seals from captive surrogates. Oikos.

<u>M.L.</u> conceived the study design, collected data, performed data analysis and drafted the manuscript. M.S. was involved in data collection, data analysis and writing. R.M. was involved with data collection and writing. A.T. was involved in data analysis and writing. D.H., R.H. and D.S. were involved in study design, data collection and writing.

Abbreviations

- COT Cost of transport
- sSMR Mass-specific standard metabolic rate
- sAMR-Mass-specific active metabolic rate
- $\dot{V}O_2$ Rate of oxygen consumption
- sVO_2 Mass-specific total energy expenditure
- $s\dot{V}O_2-\text{Mass-specific rate of energy expenditure}$
- CO₂ Carbon dioxide
- Mb-Mass
- TNZ Thermo-neutral zone
- BMR basal metabolic rate
- DBA Dynamic body acceleration
- PDBA partial dynamic body acceleration
- ODBA Overall dynamic body acceleration
- VeDBA Vectorial dynamic body acceleration
- RF random forest
- GBM -gradient boosting machine
- SL super learner
- LR logistic regression
- EE energy expenditure
- DEE daily energy expenditure

Chapter 1

Introduction

At a fundamental level in biology we aim to relate processes at different organisational levels in order to understand complicated ideas such as animal fitness (Nisbet et al. 2000). Fitness defines an animal's ability to survive and reproduce in its given environment (Orr 2009), and energetics is the study of how animals acquire their food and how they utilise that food to maximise fitness (Boyd 2002). The study of energetics allows us to learn how daily or seasonal activities are organised, how they are affected by life history or environment and the consequences of how changes to these affect the individual or population. Animal fitness (survival) is dictated by how successful an animal is at obtaining energy and using it for three key processes; maintaining body function, reproduction and growth (Figure 1.1; Costa 2002). Animals source energy from fuel (in the form of food), the process of which has its own energetic costs (Boyd 2002). A balance must be maintained between the energy used to find fuel with the energy the fuel provides. As the energy storage capacity of an animal is limited, the rate at which energy is burned is fundamental to fitness (Halsey 2011). Metabolic rate refers to the rate at which the energy is used, and it can be used to calculate how much energy (food) an animal needs to survive. As air-breathing diving vertebrates, fur seals and sea lions (otariids) have unique constraints on finding and capturing food in the marine environment (Williams and Yeates 2004). Food is sourced through diving, where otariids are not only resisting tonnes of hydrostatic pressure (particularly for the deep divers), but they must also contest with the physical forces of buoyancy and drag (Butler and Jones 1997). They must also regularly resurface to breathe and replenish oxygen stores in their bodies (Williams 1999). To extend dive durations, otariids switch between physiological processes whilst diving, only allowing oxygen supplies to the bodies vital organs (Williams et al. 2004b, Ponganis et al. 2011). The aquatic lifestyle presents thermoregulatory challenges for otariids as water conducts heat twenty-five times faster than air (Hind and Gurney 1997). One response to this challenge can be to raise the metabolic rate, though this can translate into a need for greater amounts of food, thereby influencing foraging strategies (Williams et al. 2004a).

Measuring the energetic cost of activity of wild animals can be difficult, particularly for large animals that may be difficult to handle or observe (Brown *et al.* 2013). However, because of their relationship with the land, regularly returning to breed and rest, otariids provide a unique opportunity to track their movement and energy expenditure using animal-borne devices (Crossin *et al.* 2014). Accurately estimating energy expenditure requires that the activity type, and the energetic cost of that activity, be known (Jeanniard-du-Dot *et al.* 2016b). Therefore, there has been considerable effort to identify and validate new methods and technologies that can identify the behavioural state of an animal and estimate the energetic cost of that activity (Ponganis 2007, Wilmers *et al.* 2015). Accelerometers have been shown to measure both behavioural and energetic variables in a range of terrestrial and marine animals, thereby offering a potential solution to measuring the time-energy budget of wild otariids. Therefore, the goal of this thesis was to understand the way in which otariids organise their energy expenditure, understand how it influences their interactions with the environment and investigate its potential to influence fitness using an animal-borne device – the accelerometer.



Figure 1.2.1 Conceptual model of energy flow through a typical mammal. Adapted from (Costa 2009).

1.1. Study species

The otariids of southern Australia - the Australian fur seal (Arctocephalus pusillus doriferus) (Warneke 1979), the New Zealand fur seal (Arctocephalus forsteri) (Crawley and Warneke 1979) and the endangered Australian sea lion (Neophoca cinerea) (Marlow 1975) present an interesting case of how energetics and behaviour potentially influence foraging strategies. With few predators and a range of prey, these three species dominate the ecosystems in which they reside. All three species were hunted to near extinction in the 1900's (Kirkwood and Goldsworthy 2013) and since the ban on sealing in Australia, each species has recovered, but at very different rates. The New Zealand fur seal has had the most rapid rate of recovery, currently estimated to be around pre-sealing population size and their numbers continue to increase and their range expand (McIntosh et al. 2014, Shaughnessy et al. 2014). In Western Australia the population may be nearing capacity (~17,200; Campbell et al. 2014) while in South Australia the population is estimated at nearly six times that of Western Australia (~97,200; Shaughnessy et al. 2014). Australian fur seal numbers are estimated at $\sim 22,000$ pups and the population continues to increase, albeit at a slower rate than the New Zealand fur seals (Kirkwood et al. 2010, McIntosh et al. 2014). In stark contrast, Australian sea lions are currently listed as endangered and total population numbers are estimated at ~14,300 (Goldsworthy et al., 2009). This follows the global trend of large-bodied, benthic foraging sea lion declines (Costa et al. 2004, Goldsworthy et al. 2009). Through the study of their energetics some of the difference in recovery rates may be understood.



Figure 1.2.2 Population distributions of otariids in Australia. A) Australian fur seal; B) New Zealand fur seal; C) Australian sea lion.

The different foraging and thermoregulatory strategies used by the three species incur different energetic costs. New Zealand fur seals are primarily epipelagic divers, often foraging off the continental shelf (Harcourt *et al.* 2002, Page *et al.* 2006, Baylis *et al.* 2008a), while Australian fur seals and Australian sea lions forage near shore, close to the benthos on the continental shelf (Arnould and Hindell 2001b, Costa and Gales 2003). Benthic foraging is an energetically expensive strategy and is commonplace for the generally larger sea lions, but is unusual for smaller fur seals. Australian fur seals occupy a geographic range that overlaps with that of the New Zealand fur seal. Competition for pelagic resources may have driven the adoption of a benthic foraging strategy, and subsequent increase in body size (Page *et al.* 2005a).

Despite similar foraging strategies, Australian fur seals spend less time diving at sea than Australian sea lions, likely due to phylogenetic differences. Fur seals must spend time at sea grooming their fur in order to remain insulated (Liwanag *et al.* 2012a), whereas sea lions rely on their blubber layer to stay warm (Liwanag *et al.* 2012b). As epipelagic foragers, New Zealand fur seals predictably spend less time diving at sea than the Australian sea lion and Australian fur seals (Harcourt *et al.* 2002, Page *et al.* 2005b), likely encountering more prey at shallower depths. This is perhaps a contributing factor in their healthy recovery. It is hypothesised that benthic divers operate at or near their physiological capacity, which leaves little room for switching strategies with changes in prey availability (Costa *et al.* 2004, Arnould and Costa 2006). By contrast, pelagic foragers can alter their foraging strategy by diving deeper or longer should their prey change to occupy these habitats (Costa *et al.* 2001).

1.2. Energy budgets

Successful animals will gain more energy than is required and invest the excess into growth and reproduction (Williams *et al.* 2004b, Williams *et al.* 2015). If food is plentiful and an animal is able to satisfy the energy demands of basic body functions then they may use their excess resources to enhance growth and reproduction, for example by producing larger pups (Boyd 2002). However, when food is scarce animals must work harder to find food, neglecting growth and reproduction (Williams *et al.* 1996). Developing energy budgets can be a useful method of monitoring the health of a species at the individual

or population level. These consist of two primary processes: energy gain and energy expenditure. Energy budgets can demonstrate how hard an animal is working to maintain a positive energy balance by showing how much time is invested in finding food, and monitoring if and how this changes over time. Energy gain can be estimated through the amount of food ingested, and energy expenditure is most accurately measured with metabolic rates that vary between and within species (Costa and Williams 1999).

1.2.1. Basal metabolic rate

Basal metabolic rate (BMR) accounts for most energy use when an animal is at rest, and is primarily influenced by body mass, accounting for up to 90% of variation (McNab 2008, Halsey *et al.* 2009b, Dalton *et al.* 2015). The rest of the variation in BMR can be accounted for by habitats, type of food consumed (omnivore, herbivore, carnivore), climate, substrate and reproduction strategy (McNab 2008). Due to their foraging in the marine environment, otariids generally have a much higher BMR than terrestrial mammals of equal size as a consequence of water conductivity, density and viscosity and a carnivorous diet (Lavigne *et al.* 1986, Williams *et al.* 2001). This is largely from the need to maintain body heat in cold water (Scholander *et al.* 1950) and otariids often employ behavioural thermoregulation to compensate (Liwanag *et al.* 2009 - discussed below, Liwanag 2010). Concurrently, carnivory and the heat increment of feeding (HIF) lead to a higher metabolic rate due to the high energetic demands of processing prey (Rosen and Trites 1997, Williams *et al.* 2001, Rosen *et al.* 2015).

1.2.2. Activity

Though activity in its entirety only makes up a small proportion of an animal's total metabolic rate, it can explain the largest amount of its variation (Dalton *et al.* 2015), as maintenance costs vary little, while activity costs are fluid. For otariids, activity costs outside the breeding season come primarily from foraging and requires the energy derived from food to be proportional or greater than the energy expended (Rosen and Trites 2002, Williams *et al.* 2004b). Thus, the harder an otariid has to work to find food, the more food they need to maintain the energy output (Staniland *et al.* 2007). The cost of finding energy can be largely accounted for by two components: the cost of travelling to the foraging destination, generally measured as the cost of transport (COT) (Williams 1999, Rosen and Trites 2002) and the energy expended from searching for, capturing and handling prey (Boyd 1997, Thompson and Fedak 2001).

1.2.3. Cost of transport (COT)

COT is a useful measure of energy use across species as it is a measure of the efficiency of the transport process (Feldkamp 1987). COT is estimated from swimming speed and changes depending upon the method of propulsion and positioning in the water column (Rosen and Trites 2002). Different swimming styles lead to different travel strategies and energetic outputs. Surface swimming is energetically expensive and otariids reduce this cost by using intermittent locomotion (porpoising; Au and Weihs 1980, i.e. burst-and-glide; Gleiss *et al.* 2011a) and by remaining in the water column where they are neutrally buoyant (Sato *et al.* 2013). Phocids (true seals) and otariids (fur seals and sea lions) use different methods of propulsion. Phocids propel using their hind flippers whereas otariids aquafly using their

front flippers for propulsion while steering with their hind flippers (Fish 2000). Different travel speeds can be achieved through using the different swimming strategies and, though it is not constant, there is a general relationship between COT and velocity (Rosen and Trites 2002). Minimum COT is the range of speeds at which a marine mammal transports itself through the water column with the least amount of energy (Schmidt-Nielsen 1972). COT varies by species and individual and may also influence prey choice of a species, as a balance must be maintained between energy intake and the distance covered in order to get that energy (Staniland *et al.* 2007).

1.2.4. Diving

One of the main adaptations of seals to the marine environment is their ability to hold their breath and dive for extended periods of time. This adaptation has developed through persisting on a diet of mobile aquatic and semi-aquatic animals, that in order to capture require seals to have exceptional speeds and complex manoeuvres (Bowen *et al.* 2002). Seals make a number of behavioural adaptations and physiological acclimations to maintain their underwater foraging behaviour (Williams *et al.* 2015). Their long dive durations can be explained by extreme bradycardia, which lowers the metabolic rate, and the alteration of stroking frequency supported by the animal's buoyancy (Ponganis *et al.* 1991, Williams *et al.* 2004b).

Positively buoyant phocids begin their dive with an increased stroking frequency in order to overcome the effect of buoyancy (Davis and Weihs 2007). Negatively buoyant phocids must work predominantly at the end of the dive, continuously stroking on the dive ascent (Maresh *et al.* 2015). When neutrally buoyant, or close to it, phocids can reduce their stroking frequency and instead use long bouts of gliding, where they are effectively using no additional energy (Davis *et al.* 2001). These same behavioural adaptations are apparent in other marine mammals, where bottlenose dolphins (*Tursiops truncatus*) stroke continuously on the dive decent, and use some stroking with long bouts of gliding on the ascent (Williams 2001). However, it is yet to be investigated as to whether this same behavioural strategy exists for otariids.

While diving, marine mammals elicit a dive response where longer dives correspond to a lower metabolic rate (Davis and Williams 2012). The dive response slows heart rate (bradycardia) and blood flow, thus oxygen delivery, to the vital organs, while the non-vital organs have blood flow restricted, resulting in the retention of oxygen stores for longer (Ponganis *et al.* 2011). At the cessation of a dive seals utilise tachycardia, large tidal volumes, and high respiratory frequencies to oxygen load at the surface very quickly (Williams *et al.* 1991, Reed *et al.* 1994), while simultaneously removing excess carbon dioxide from the system (Fahlman *et al.* 2008a). Because of these adaptations, seals generally have much shorter surface durations than dive durations and spend most their time at sea submerged (Boyd and Croxall 1996).

The time that an air-breathing animal can remain underwater whilst using oxygen stored in the body is the aerobic dive limit (ADL) (Davis and Weihs 2007) and this is supported by reducing the demand for oxygen through the suppression of non-essential body functions, such as digestion, while diving (Costa *et al.* 1989, Sparling and Fedak 2004, Fahlman *et al.* 2008b). After a significant depletion of oxygen stores the body becomes reliant on the anaerobic metabolism, which is undesirable, as after long periods the muscles in the body will cease to function (McIntyre *et al.* 2002). This is because throughout the dive otariids are producing a build-up of lactic acid in the body which restricts the muscles from performing optimally (Ponganis *et al.* 1997). Large build-ups of lactic acid result in longer surface periods between dives, thus reducing the efficiency of foraging at depth (Mori 1999).

1.2.5. Thermoregulation

The challenges of thermoregulation are exacerbated for otariids as a result of their transitional lifestyle between terrestrial and marine habitats. Metabolic adjustments are required by animals when their body temperature falls outside their thermo-neutral zone (TNZ). The TNZ for a given animal is the range of temperature within which the animal can maintain their core body temperature without making any adjustments to their metabolic rate (Wood 1991). Conductivity of water causes animals to lose heat at a higher rate than air, thus otariids are losing energy faster whilst foraging their terrestrial counterparts (Matsuura and Whittow 1973). Thick blubber and layers of fur help to regulate this loss (Scholander *et al.* 1950), along with a variety of other mechanisms including; an elevated metabolic rate (Liwanag *et al.* 2009), vascular specialisations (Mellish *et al.* 2004) and increasing the heat production associated with assimilating food (Rosen and Trites 1997). The different strategies used by fur seals and sea lions to thermoregulate influence their overall energy use.

Fur seals rely on a thick layer of fur to thermoregulate, as the blubber layer they maintain is metabolically inert and used primarily for energy storage (Liwanag *et al.* 2012a, Dalton *et al.* 2014a). The fur seal strategy means that less energy is used when cold, as a layer of air trapped between their fur layers is used as insulation (Liwanag *et al.* 2012a). Behaviourally this strategy requires a significant amount of maintenance, so fur seals must spend a lot of time grooming their pelage (Battaile *et al.* 2015). This strategy aids thermoregulation in cold water by raising the metabolic rate. Metabolic rate is raised by using behavioural strategies such as jughandling and sailing, which may also be used to cool the body down (Liwanag 2010). The body is cooled in warm water by lifting the flippers out of the water to expose them to the cool breeze, while they are lifted out of cool water to limit the exposure time (Bartholomew and Wilke 1956, Liwanag 2010).

Sea lions rely on a thicker blubber layer to protect themselves from cold water (Mellish *et al.* 2007, Williams *et al.* 2007, Liwanag *et al.* 2012b) that is interspersed with layers of muscle (Mellish *et al.* 2004). Sea lion blubber forms in two layers, one for energy storage which generally maintains a constant thickness throughout the year, and one for thermal insulation, which fluctuates with the changing temperature (Williams *et al.* 2007). Rather than using behavioural strategies to cool down, sea lions take advantage of their blubber as living tissue, bypassing it through perfusion in warm temperatures (Meagher *et al.* 2008), and relying on a thicker layer of blubber in cool temperatures.

1.2.6. Annual cycles

Metabolic rates vary during different seasons and annual events such as breeding and moulting (Rosen and Renouf 1995, Dalton *et al.* 2015). Generally, otariids have three major physiological changes that

occur over the course of a year; an annual moult (during which time pregnant females give birth), a period of lactation and breeding; and a long period of foraging to build energy reserves for the moult (Stirling 1971, Costa and Gentry 1986). Most otariid species (excluding the unique Australian sea lion) pup and breed in late spring and early summer, moult in summer and forage during autumn and winter (Costa 1991b, Trites and Antonelis 1994). Australian sea lions are an exception, they have a 17.5 month breeding cycle, can moult at any time of the year, and feed intermittently throughout their breeding period (Higgins 1993, Gales *et al.* 1994). Estimating the energetic demands from these different annual cycles is difficult due to the large amount of inter- and intra- species variation, but is important for developing accurate energy budgets.



Figure 1.2.3 Moulting, breeding, and pupping timeline of Australian fur seals, New Zealand fur seals and Australian sea lions.

Seasonally replacing hair is vital for seals to maintain a pelage useful for deep diving (Ling 1970). Phocid moult is catastrophic (occurring rapidly) and as a result generally occurs in summer to maximise skin surface temperature for the promotion of hair growth (Paterson *et al.* 2012), since they are unable to efficiently thermoregulate (Feltz and Fay 1966). The otariids moult is less catastrophic, and while still generally occurring in summer, occurs over a longer duration and does not restrict the animals from using the water (Trites and Antonelis 1994). However, the effect the moult has on metabolic rates varies with species and reproductive status. For example, a comparison of northern hemisphere phocids reveals that for grey seals (*Halichoerus grypus*) metabolic rate is highest during winter (Boily and Lavigne 1997) while for harp seals (*Pagophilus groenlandicus*) it is highest in summer (Renouf and Gales 1994). Nonreproductive California sea lions (*Zalophus californianus*) showed a significant increase in resting metabolic rate during the moult, while reproductive animals did not display any differences (Williams *et al.* 2007). The growth of new hair would increase the metabolic rate (Boyd *et al.* 1993), while a decrease in metabolic rate would delay fat loss while hauled out (Beck *et al.* 2003a).

Male and female otariids occupy vastly different roles during reproduction and this is exemplified in their extreme sexual dimorphism. Each year adult females must mate, gestate, give birth and wean a pup, in between moulting and foraging for food (Arnould 1997). Males compete for females and in order to

become dominant must be larger than other males from the same colony (Boyd and Duck 1991). During the breeding period males will remain hauled out to defend their territory, while after a short period on land following birthing, otariid females will intermittently forage and lactate (Costa and Trillmich 1988). For female fur seals and sea lions there have been no reported effects of pregnancy (Williams *et al.* 2007) or lactation (Costa and Trillmich 1988) on metabolic rate. Territorial males exhibit predictable mass loss during this time, presumably coupled with a lower metabolic rate (Boyd and Duck 1991)

1.2.7. Sex

Varying metabolic rates, and thus different diving abilities, may also arise as a result of sex. Female seals have higher mass-corrected total oxygen stores and higher rates of oxygen consumption than males (Rosen and Renouf 1995, Weise and Costa 2007). Female Californian sea lions and Steller sea lions (*Eumetopias jubatus*) have greater mass-specific total oxygen stores than males (Richmond *et al.* 2006, Weise and Costa 2007) and higher mass-specific metabolic rates (Hurley and Costa 2001). Diving differences in male and female phocids vary across species, where some males dive deeper and longer than females (Le Boeuf *et al.* 2000, Page *et al.* 2005b), there are no sex difference in others (i.e. harbour seals (Phoca vitulina); Tollit *et al.* 1998) and for some females dive deeper and longer than males (i.e. southern elephant seals (Mirounga leonina); Hindell *et al.* 1991). Very few male otariids have been tagged in the wild, and as such the sex differences within this group are not well understood. One study on New Zealand fur seals showed that males dived deeper and longer than females (Page *et al.* 2005b). Any differences observed are suggested to arise from the need of the female to compensate for a small body size, but may also be as a result of the extreme sexual dimorphism apparent in seals (Beck *et al.* 2003b).

1.2.8. Development stage

Pups and juvenile seals generally have higher energetic costs than adults due to the cost of growth (Donohue *et al.* 2000), though this is not consistent across species. Steller sea lion, grey seal and harbour seal juveniles have higher mass-specific metabolic rates than adults that declines as they approach sexual maturity (Rosen and Renouf 1995, Boily and Lavigne 1997, Richmond *et al.* 2006). In one study it was reported that the resting metabolic rate of juvenile and adult Californian sea lions did not differ significantly (Liwanag *et al.* 2009). It is expected that otariid pups develop adult oxygen stores and metabolic rates slowly as they remain with their mothers for an extended period (Fowler *et al.* 2007b). While phocid pups tend to develop the physiological capacity to dive earlier due to the rate at which weaning occurs, where phocid pups gain energy from their mothers rapidly due to the limited investment in parental care (Noren *et al.* 2005, Burns *et al.* 2007).

1.2.9. Summary of inter- and intraspecific differences

While this review is not comprehensive, it does serve to highlight the significant variation that exists between and within otariid species. Understanding how energy expenditure varies for animals during different annual and life stages allows us to develop accurate energy budgets. The evidence highlighted so far suggests that, where possible, energy budgets must be unique to species across their annual and life stages. Accurately measuring the metabolic rate of otariids in the wild is a difficult task as they travel great distances and dive to incredible depths to find their food. The following section outlines some advantages and disadvantages of methods currently available for measuring otariids energetics.

1.3. Methods of measuring behaviour and energetics

Gaining accurate information on metabolic rates of free-living animals has been investigated using a number of methods, including; heart rate monitoring, doubly labelled water, respirometry, stroke rate and more recently accelerometry, each with their own advantages and disadvantages. The challenge is to find a bio-logging tool that both accurately measures energy expenditure while being the least invasive to the animal. Over time bio-logging devices have become miniaturised but can store exponentially more data, thus increasing the overall detail and accuracy they can provide. Three of the most widely used methods of measuring field energy expenditure are outlined here, along with a discussion of the 'gold-standard' method – respirometry.

1.3.1. Respirometry

As food is metabolised by the body it is changed into different forms of energy, most of which is wasted in the form of heat. The rate at which the heat is expelled from the body is the metabolic rate and can be measured directly using calorimetry (McLean and Tobin 1987). Calorimetry requires the target species to be placed in a sealed chamber lined with sensors that measure the heat expended from the animal. While this is the most accurate measurement of metabolic rate available, it is impractical and costly. Respirometry affords a practical solution, measuring metabolic rate instead through gas exchange.

Being diving air-breathing vertebrates, otariids offer a unique opportunity to measure animals after exercise as they must return to the surface to breathe. Otariids can be trained to swim beneath the water, and breathe into a floating hood at the surface which is connected to a respirometer (Rosen *et al.* 2016). The respirometer measures the rate at which the otariids are consuming oxygen and/or carbon dioxide which can be converted into a rate of energy expenditure (Withers 2001). Respirometry is generally considered the 'gold-standard' of measuring energy expenditure (Halsey 2011), but is often limited to the laboratory. A noteworthy exception to this is Kooyman's (1966, 1985) experiments with freely diving Weddell seals (*Leptonychotes weddelli*). Seals were relocated to an isolated ice hole which seals were compelled to return to breathe as its proximity to another breathing hole was too great. The ice hole was covered by a respirometer and the gas exchanges of seals were collected when they surfaced to breathe. These experiments have since been repeated with Weddell seals (Ponganis *et al.* 1993, Williams *et al.* 2004b), but no other wild seal has had their energy expenditure through diving measured via respirometry.

Other respirometry studies measuring the energy expended from activity have been limited to seals held in captivity. Measuring seals in captive and semi-captive environments allows for experimental manipulation such that a number of physiological parameters can be investigated including; the metabolic cost of diving (Fahlman *et al.* 2008a), the dive response (Hindle *et al.* 2010), cost of transport (Rosen and Trites 2002) and foraging efficiencies (Sparling *et al.* 2007) among many others (e.g. Gerlinsky *et al.* 2014a, Dalton *et al.* 2015, Goundie *et al.* 2015). However, respirometry studies are constrained by the need for the animal to return to or remain in the respirometer hood. This means that respirometry studies can only be used for specific activities or specific time periods (Fort *et al.* 2011), and have limited applicability wild animals. Therefore, we seek to identify a bio-logging method that allows for accurate measurement of energy expenditure over long periods, related to the energy expended via respirometry.

1.3.2. Accelerometry

Locomotion may be used to predict the metabolic rate of free-ranging otariids (Jeanniard-du-Dot *et al.* 2016b). Measuring field metabolic rate via accelerometry relies on the theory that above basal metabolic rate, movement can account for the largest variation in metabolic rate (Karasov 1992) and is thus correlated with energy expenditure (Wilson *et al.* 2006). Accelerometers measure the acceleration of an animal on three axes – heave, surge and sway – that can be summed to give an overall estimation of energy expenditure (Halsey *et al.* 2011a). So far, two types of estimations have been derived – vectorial and overall dynamic body acceleration to calculate static acceleration (DBA) is derived by applying a running mean over the axes of acceleration to calculate static acceleration (gravity) and removing this from the raw acceleration (Shepard *et al.* 2008a). Then, either the absolute dynamic acceleration is summed to give overall dynamic body acceleration (ODBA) or the square root of the sum is taken to give vectorial animals measured via respirometry (Qasem *et al.* 2012).



Figure 1.2.4 Accelerometer direction when fitted to a sea lion wearing a custom made harness.

The relationship between DBA and energy expenditure in marine mammals has thus far been inconclusive (Fahlman *et al.* 2008b, Volpov *et al.* 2015b), and is by no means as strong as for terrestrial animals (Halsey *et al.* 2009b). The key assumption of the relationship between DBA and oxygen consumption is that as animals increase their rate of movement they also increase their rate of energy expenditure. Accelerometers allow the entire movement of a dive to be captured, thereby has the

potential to break down the components of a dive and their associated metabolic costs (Fahlman *et al.* 2008b, Halsey *et al.* 2011a). Therefore, these types of behaviours can be built into energetics models to make a more accurate estimate of a otariids energy expenditure over time (Fahlman *et al.* 2013). Recently this method has been applied to wild fur seals where it was demonstrated that VeDBA was linearly and significantly related to energy expenditure as measured by DLW (Jeanniard-du-Dot *et al.* 2016b). However, it has since been demonstrated that this relationship falls into the "time-trap", where the relationship found was simply the cumulative energy expended over time, rather than the energy measured via accelerometry (Halsey 2017a). Further evidence of the lack of relationship between ODBA and energy expenditure has been provided from experimental trials with Steller sea lions, where it was demonstrated that the dive phase was highly influential (Volpov *et al.* 2016). The highly variable results of this relationship warrants further investigation for clarity.

1.3.3. Stroke rate

Another potential proxy for energy expenditure in otariids – stroke rate – is derived from accelerometry (Williams et al. 2004b). The principal assumption behind this relationship reflects that of DBA whereby it is assumed that the primary energy expenditure is derived from muscle movement. However, the energy expended over the course of a dive will differ for a marine mammal depending on the depth, duration and dive phase (Volpov et al. 2016). During a dive, most of the movement comes from the forward propulsion from stroking. During diving four species of marine mammal; blue whale (Balaenoptera musculus), bottlenose dolphin, Weddell seals and elephant seals were observed using a continuous stroking action to begin their dive, followed by a prolonged period of gliding to the maximum depth of that dive (Williams et al. 2000). The ascent for each species was characterised in the same manner whereby they all used sequential, large-amplitude strokes to begin the ascent, followed by stroke-andglide swimming for the remainder. While there was a linear relationship, it is likely that not all aspects of movement from the dive are captured with stroke rate. For example, while swimming up or down may produce the same number of strokes, the metabolic cost associated with the different directions changes (Sato et al. 2003). As a buoyant animal, particularly after long periods of foraging and mass gain, the energy required to swim down is far greater than in times of scarcity (Sato et al. 2003). A positive linear relationship was shown between total strokes and total oxygen consumption over a dive in wild Weddell seals (Williams et al. 2004b) and for other phocid species swimming in a flume (Davis et al. 1985, Fish et al. 1988). These relationships have since been called into question due to falling into the same "timetrap" as the previously mentioned relationships of ODBA with energy expenditure (Halsey 2017a). Therefore, the number of strokes cannot be used a predictor of energy expenditure, but the relationship of stroke rate with a rate of energy expenditure is unclear.

1.4. Research objectives

The goals of this thesis are threefold:

1) To fill the current knowledge gap on the basic physiology of three Australian seal species.

To-date there have only been physiological measurements made of wild Australian sea lions with doublylabelled water (Costa and Gales 2003, Fowler *et al.* 2007b). While this method provides a good estimate of energy expenditure in the wild, it is limited to short time frames and cannot identify the cost of different behaviours. Instead I propose, using a sample of captive Australian fur seals, Australian sea lions and New Zealand fur seals, to measure via respirometry the energy expended from rest and activity. Further, I aim to identify how energy expenditure differs across age classes, sexes and how it varies with annual life cycles and temperatures.

2) To validate the use of accelerometers to measure behaviour and energy expenditure

To fully comprehend how animals may respond to changes in their environment both their behaviour and their physiology should be monitored (Cooke *et al.* 2014). Time-energy budgets can only be built when both the activity and the energetic cost of that activity are known. Accelerometers afford an opportunity to use a single device to measure both aspects of an animal's ecology. Using captive animals, I aim to validate the use of accelerometers to distinguish between groups of ecologically important behaviours. I also aim to validate data from accelerometry as a proxy for energy expenditure.

3) To build time-energy budgets of wild otariids using accelerometers

Conducting research with captive animals can be useful in furthering our knowledge of a species. This thesis aims to take this further by developing a model that can be generalised across individuals of a range of species. I achieve this goal by developing models on a range of individuals of different species, masses, ages and sexes. The models are validated on a sample of juvenile Australian fur seals.

Chapter 2

Intrinsic and extrinsic influences on the standard metabolic rates of three species of Australian otariid

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Abstract

The study of marine mammal energetics can shed light on how these animals might adapt to changing environments. Their physiological potential to adapt will be influenced by extrinsic factors, such as temperature, and by intrinsic factors, such as sex and reproduction. We measured the standard metabolic rate (SMR) of males and females of three Australian otariid species (two Australian fur seals, three New Zealand fur seals and seven Australian sea lions). Mean SMR ranged from 0.47 to $1.05 \ IO_2 \ min^{-1} \ which$ $when adjusted for mass was 5.33 to 7.44 ml O_2 min^{-1} kg^{-1}. We found that Australian sea lion mass$ $specific SMR (sSMR ml O_2 min^{-1} kg^{-1}) varied little in response to time of year or moult, but was$ significantly influenced by sex and water temperature. Similarly, Australian and New Zealand fur sealssSMR were also influenced by sex and water temperature, but as well by time of year (pre-moult, moultor post-moult). During the moult fur seals had significantly higher sSMR than at other times of the year.For both groups females had higher sSMR than males, but sea lions and fur seals showed differentresponses to changes in water temperature. Fur seals sSMR increased with increasing water temperaturewhile sea lions sSMR decreased with increasing water temperature. There were no species differencewhen comparing animals of the same sex. Our study suggests that fur seals have more flexibility in theirphysiology than sea lions, perhaps implying that they may be more resilient in a changing environment.

Key words

Standard metabolic rate, otariid, sex, moult, water temperature

2.1. Introduction

Predicted global climate change is already altering the marine environment and will subsequently affect the animals that live and hunt within its bounds (Simmonds and Isaac 2007). Some of the changes expected include increasing ocean temperatures and changes to seasonal oceanic processes that will likely affect the distribution of fish assemblages within the marine environment (Learmonth *et al.* 2006, Schumann *et al.* 2013). Marine mammals may be particularly susceptible to these changes if their prey distribution alters such that they have to travel further or dive deeper to obtain food (Staniland *et al.* 2007), or if the marine environment warms to an extent that they cannot thermoregulate effectively (Boyles *et al.* 2011). Thus, in order to predict how changes in environmental conditions might impact on pinnipeds it is important to understand how different groups utilise their energy stores over a range of environmental conditions (Canale and Henry 2010). Understanding how much flexibility that marine mammals have in order to adapt to the changing conditions can be, in part, met through studying their energetics (Geiser and Turbill 2009, Canale and Henry 2010).

The study of energetics provides information about the needs of animals as well as the cost of satisfying those needs (Williams and Yeates 2004). Survival requires an animal to maintain an overall positive energy balance, satisfied by obtaining more energy than is expended. Energy expenditure is most accurately estimated by determining metabolic rates, and these can vary over seasons and years with body mass accounting for most of this variation (Kleiber 1947, McNab 2008). However, intrinsic factors such as reproduction (preparation for and recovery from the energetic demands of the breeding season), moult and sex, and extrinsic factors such as temperature and photoperiod can also contribute to some of this variation. These factors have been investigated in a wide range of phocid (e.g. Rosen and Renouf 1995, Boily and Lavigne 1997, Ochoa-Acuña *et al.* 1998, Sparling *et al.* 2006), and otariid seals (e.g. Costa and Gales 2003, Williams *et al.* 2007) but have not shown any consistent relationships among species.

Harbour seals (*Phoca vitulina*) demonstrate sex and age variation with metabolic rates declining with age, females faster than males, and they experience metabolic depression during pre- and post-moult stages (Rosen and Renouf 1995). In contrast, grey seals (*Halichoerus grypus*) have their highest metabolic rate during winter and they increase, rather than depress, their metabolic rate during the moult (Boily and Lavigne 1997). Within otariids there appears to be clear seasonal patterns in metabolic rate of fur seals (Dalton *et al.* 2015), though no effect of reproduction or season has been found for sea lions (Williams *et al.* 2007). The processes that underlie these variations in response to changing environmental conditions are not well understood, and it is clear that the responses vary greatly between and within pinniped species.

Fur seals and sea lions differ greatly in their thermoregulatory strategies. Fur seals rely on a two thick layers of fur to thermoregulate, trapping a layer of air between their fur layers to support its insulation (Liwanag *et al.* 2012a). The fur seal blubber layer is metabolically inert and is primarily used for energy storage (Liwanag *et al.* 2012a, Dalton *et al.* 2014a). Sea lions on the other hand, rely on a thick blubber layer interspersed with layers of muscle (Mellish *et al.* 2004) to protect themselves from cold water

(Mellish *et al.* 2007, Williams *et al.* 2007, Liwanag *et al.* 2012b). Sea lions maintain two blubber layers, one for energy storage which maintains a constant thickness throughout the year, and one for thermal insulation, which responds to changes in temperature (Williams *et al.* 2007).

The three otariid species that occupy Australian waters present an interesting comparison of how marine mammals may respond to ecosystem changes, as they have different reproductive cycles, thermoregulatory methods and foraging strategies. The Australian fur seal (*Arctocephalus pusillus doriferus*) and the New Zealand fur seal (*A. forsteri*) have an annual breeding and moulting cycle typical of pinnipeds (Fig. 2.1; Goldsworthy and Shaughnessy 1994, Gibbens and Arnould 2009). By contrast the Australian sea lion (*Neophoca cinerea*) breeds asynchronously every 17-18 months (Higgins 1993, Gales *et al.* 1994) and has an extended moult that can occur any time of the year. Both the Australian fur seal (Arnould and Hindell 2001a, Kirkwood *et al.* 2006), and the Australian sea lion are predominantly benthic foragers (Costa and Gales 2003, Lowther *et al.* 2013), while the New Zealand fur seals are typically pelagic foragers (Harcourt *et al.* 2002).

The habitat of the Australian fur seal and the Australian sea lion do not overlap, but the New Zealand fur seal occurs across both the feeding and breeding ranges of the other two species (Page *et al.* 2005a, Campbell *et al.* 2014). The ranges of the Australian fur seal and the New Zealand fur seal are currently expanding as they begin to reoccupy territory they held before commercial sealing (Goldsworthy *et al.* 2003), whereas the Australian sea lion is listed as endangered and the population continues to decline (McIntosh *et al.* 2013b). Through investigating how marine mammals occupying similar habitats but using different reproductive and foraging strategies vary their primary energy expenditure over the course of a year we can begin to understand how they might respond to environmental changes. Therefore, it was the aim of this study to explore the intrinsic and extrinsic influences on metabolic rate in a sample of fur seals and sea lions.

2.2. Materials and Methods

2.2.1. Animals

We conducted experiments to measure the metabolic rates of captive seals (N=12) in three Australian marine facilities: Dolphin Marine Magic, Coffs Harbour (RF1: 30°17'S, 153°8'E); Underwater World, Sunshine Coast (RF2: 25°40'S, 153°7'E); and Taronga Zoo, Sydney (RF3: 33°50'S, 151°14'E). Experiments were conducted at various times of year over from 2013 to 2015. Due to logistical constraints it was not possible to measure all seals in the same month of the same year (data collection periods are shown in Fig. 2.1). Rather, for fur seals we ensured sampling was spread over the year but included each significant stage of their annual cycle (analogous to moult, post-moult and prior to the moult, but before breeding; Fig. 2.1). Australian sea lions were measured at the same time as the fur seals as we could not determine their moulting and breeding cycles. During each visit to the marine facility the animals were measured between one and four times. We used three New Zealand fur seals, two Australian fur seals and seven Australian sea lions, all of which were not reproducing at the time of

experiments, were on permanent display at their respective facilities, and were cared for under the husbandry guidelines of that facility. The study was approved by Macquarie University ethics committee (ARA-2012_064) and Taronga Conservation Society Australia ethics committee (4c/10/13). All Australian sea lions that participated in the study were born as a part of an ongoing captive breeding program in Australian aquaria. Whereas all fur seals came into captivity as juveniles after having been found in poor health or injured and were considered unsuitable for release back into the wild after rehabilitation. Fur seal ages were estimated from their size and condition when they were introduced to their facility and are now all subadults or adults. Seals were weighed once per week as a part of their normal routine.

2.2.2. Metabolic rate measurements

We measured standard metabolic rates (SMR) of seals using open-flow respirometry. Standard metabolic rate was used as seals were measured in water and they did not adhere to all the standards of Kleiber for measuring basal metabolic rate (Kleiber 1975, Hurley and Costa 2001). Seals had not fed for at least 10 hours prior to each trial to ensure they were post-absorptive (Rosen and Trites 1997), and no animals were pregnant or lactating. Seals were quiescent (not sleeping) during measurement, and reached steady-states of oxygen consumption in five minutes or less. As measurements of SMR were taken in water and for a short period of time, they were not considered to be true representations of resting or basal metabolic rate. Seals were measured early in the morning before they had become active (i.e. swimming) and only participated in trials if they were found to be dry in their enclosure. Measurements of metabolic rate were recorded for up to 15 minutes, with the lowest, consistent three minutes (minimum) being used for analysis.

We measured SMR when seals were sitting upright and still in water under a moulded acrylic hood (80 L). This behaviour was reinforced with small amounts of food (fish and squid), which was reduced as each seals capacity to remain inactive improved with training. This amount of food would not have influenced metabolic rate (Rosen and Renouf 1997, Rosen et al. 2015). The hood was connected to an open-flow respirometry system (Sable Systems International, Inc., Henderson, NV, USA) where air was pulled from the hood with a Sable Systems Mass Flow pump at an adjustable flow rate ranging from 300-350 l min⁻¹. We adjusted and monitored the flow for each individual to ensure that the oxygen inside the hood remained above 20%, carbon dioxide was scrubbed from the sample and therefore not monitored. A continuous sub-sample was drawn into the analyser from the pump at ~1200 ml min⁻¹, pushed through the oxygen analyser and measured for water vapour then dried (magnesium perchlorate), scrubbed of carbon dioxide (soda lime) and before entering an FC-1 oxygen analyser. To ensure that the measured CO₂ and water vapour fluctuations did not exceed 1% and 5% respectively, the scrubbers were monitored using the built-in CO₂ analyser and an external water vapour analyser. The percentage of oxygen in the expired air was measured continuously with Sable Systems ExpeData software and recorded at five samples per second. Oxygen consumption (VO₂) was calculated using equations from Withers (1977) assuming a respiratory quotient (RQ) of 0.77 (Feldkamp 1987, Boyd et al. 1995b) as the animals were fed in water the effect of digestion was assumed to be depressed.

We calibrated the system every two to three days using nitrogen (N₂) and ambient air following the method of Fedak *et al.* (1987). Nitrogen gas was passed through a flow meter at a known rate using a Sable Systems FoxBox. The predicted values of the N₂ flow were 400 ml min⁻¹ and 500 ml min⁻¹. Values were within \pm 5% of predicted values.

2.2.3. Analyses

Prior to analysis we examined the suitability of the data for analysis using linear models. We used a linear regression to investigate the relationship between mass (kg) and SMR ($l O_2 min^{-1}$). Due to the large range, mass was log-transformed, and we used mass-specific SMR (henceforth sSMR ml $O_2 min^{-1} kg^{-1}$) to make statistically relevant comparisons across fur seal and sea lion groups. We identified outliers in the continuous response variables (SMR; sSMR) using exploratory graphical techniques and removed any that corresponded to a behavioural anomaly. We also assessed collinearity-correlation among explanatory variables (mass, sex, age, moult (presence/absence), animal ID, month, ambient temperature, water temperature) via multiple pair-wise scatterplots (pair plots) (Zuur *et al.* 2009b, Zuur *et al.* 2010). We examined the response variables for normality visually using a histogram and any factor explanatory variables were tested for equal variances across the response variable (Bartlett's test).

We measured the metabolic rate of a subset of six seals (NFM1, AFM1, ASM2, ASF2, ASF3, ASF5) in the same month, one year apart and used paired t-tests to look for differences in mean mass-specific standard metabolic rate to test for a training effect. As there were no significant differences (P > 0.05) in mean sSMR for any of the six seals between the two years, training effects were not considered further.

Fur seal and sea lion sSMR data were analysed separately. We used multiple linear mixed-effects models (LME) with restricted maximum likelihood (REML) estimation to evaluate which sources of variation best explained changes in SMR (l O2 min-1) (NLME package in R; (Pinheiro et al. 2014). Using SMR as the response variable, we first ran a null model (no random effects) to find a baseline from which we could evaluate the influence of the random effect on the models. We then ran LME's with animal ID as the random effect to account for repeated measures. The predictor variables for sea lions were: mass, sex, age, month of the year, moult (absence/presence), and water temperature. We did not use ambient temperature in the models as it was highly collinearly related to water temperature, which was used in preference as the animals were measured in the water. Since month is a cyclical variable we transformed it to sine $(\sin(360/11) \times \text{month})$ or cosine $(\cos(360/11) \times \text{month})$ as in Sparling *et al.* (2006), and both were tested in the model. The predictor variables for fur seals were: mass, sex, age, species, season (premoult, moult, post-moult) and water temperature. We chose to use an information-theoretic approach to build candidate models as stepwise model selection can produce unreliable results (Whittingham et al. 2006). The models were run with all combinations of predictor variables using dredge from the R package MuMIn (Bartoń 2013). Models were ranked using "model.sel" from the R package MuMIn and Akaike model weights were used to rank the models.

Model selection was based on a combination of Akaike Information Criteria (AICc), log likelihoods (logLik) and R². The amount of variance explained by the random effect was assessed through the

difference of the marginal (fixed effect only) and conditional (all model variables) R^2 (*rsquared.glmm* function). The assumptions of homoscedasticity, normality, homogeneity and independence were investigated by plotting predicted vs fitted residuals, QQ-plots, Cleveland dot-plots and ACF plots (Zuur *et al.* 2009a). All analysis was completed in R (Version 3.1.3; R Core Development Team 2015) and values are reported as mean \pm SD.

2.3.Results

Metabolic rates measured at rest in water were collected for twelve seals at semi-regular intervals over three years (Fig. 2.1). A total of 155 measurements were collected, with 153 used for analysis as two observations were excluded as they were identified as outliers from behavioural anomalies. There was a strong positive relationship between SMR (l $O_2 \min^{-1}$) and log mass (kg) for all 12 seals expressed by the equation log(SMR) = -3.48 + 0.66 log(mass) (logLik = 57.78, R² = 0.769, p < 0.001, Fig. 2). Mean SMR for all seals ranged from 0.34 l $O_2 \min^{-1}$ to 1.31 l $O_2 \min^{-1}$ and sSMR ranged from 3.06 ml $O_2 \min^{-1}$ kg⁻¹ (Table 2.1).



Figure 2.1 Relationship between log standard metabolic rate and log mass for 12 seals. Log of metabolic rate while resting in water (SMR l $O_2 \min^{-1}$) as a function of log body mass (kg) for one female Australian fur seal (N = 13), one male Australian fur seal (N =16), three male New Zealand fur seals (N = 31), five female Australian sea lions (N = 68) and two male Australian sea lions (N = 26). Line plotted is the fitted equation: log(SMR l $O_2 \min^{-1}$) = -3.48 + 0.66(log (mass)).

2.3.1. Fur seals

Australian fur seals and New Zealand fur seals have an annual moult and breeding season that occur at similar times of year (Fig. 2.1). Age and mass were highly colinearly related for the male fur seal, therefore

only mass was included in the model. The model that best explained the variation in SMR for fur seals included season (pre-moult, moult, post-moult) and mass, and there was a large effect of animal ID (LME: AICc = -35.7; logLik = 27.8, R²(conditional) = 0.544; R²(marginal) = 0.732). There was no significant effect of water temperature, sex or species. sSMR for both males and females was lowest during post-moult (Fig. 2.3). For males, sSMR was highest during the annual moult, while for females sSMR was highest during the pre-moult (Fig. 2.3). While season was able to explain more of the variance in the model than water temperature, there was a positive linear relationship between water temperature and sSMR for each of the four fur seals (Fig. 2.4A-D).

Table 2.1 Summary statistics of standard metabolic rates (SMR) for 12 seals. Mean \pm SD of SMR ($O_2 \min^{-1}$) and sSMR ($ml O_2 \min^{-1} kg^{-1}$) multiples of BMR and the age, mass range and sample sizes for Australian fur seals, New Zealand fur seals, and Australian sea lions.

Sex	N	Age range	Mass	Total	SMR	sSMR	BMR	
		(years)	range (kg)	trials	$(l \operatorname{O}_2 \operatorname{min}^{\text{-}1})$	$(ml~O_2min^{1}~kg^{1})$	multiple	
Australian fur seal								
Female	1	17.8 - 19.1	69 - 79	13	0.49±0.06	6.63±1.04	2.0	
Male	1	15.1 - 17.1	175 - 242	16	1.05 ± 0.20	5.33±1.18	2.1	
New Zealand fur seal								
Male	3	7.5 - 14.0	47 - 161	31	0.62±0.18	6.42±1.66	2.2	
Australian sea lion								
Female	5	5.1 - 26.4	44 - 76	68	0.47 ± 0.08	7.44±1.16	2.1	
Male	2	9.0 - 14.3	108 - 177	25	0.84±0.13	5.94±1.09	2.0	



Figure 2.2. Boxplots of metabolic rate for fur seals in relation to the moult. Median, interquartile range (box) and range (bars) of mass-specific standard metabolic rate (sSMR ml $O_2 \text{ min}^{-1} \text{ kg}^{-1}$) for an Australian fur seal male (black box, N = 1) and female (white box, N = 1) and New Zealand fur seal males (grey box, N = 3) during the moult, post-moult and pre-moult periods.



Figure 2.3 Moulting, breeding, and pupping timeline of Australian fur seals, New Zealand fur seals and Australian sea lions for experiments conducted at three Australian marine facilities over three years. Shaded boxes indicate trials were conducted during that month in the respective facility. RF1 is located in a temperate to sub-tropical region, RF2 is located in a sub-tropical region and RF3 is located in a temperate region. *Fur seals*



Figure 2.4 Relationship between sSMR (ml O₂ min⁻¹ kg⁻¹) and water temperature (W_T °C) for four fur seals. A) Female Australian fur seal (sSMR = 1.12 + 0.21 × WT, logLik = -17.42, R² = 0.140, p = 0.207, n = 13). B) Male Australian fur seal (sSMR = -9.70 + 0.59 × WT, logLik = -15.63, R² = 0.683, p < 0.001, n = 16). C) Male New Zealand fur seal (sSMR = -5.99 + 0.45 × WT, logLik = -18.36, R² = 0.404, p = 0.011, n = 15). D) Male New Zealand fur seal (sSMR = 1.95 + 0.29 × WT, logLik = -14.70, R² = 0.587, p = 0.003, n = 12).

2.3.1. Sea lions

Australian sea lion moult and breeding can occur at any time of the year so it was not possible to examine the effect of season on metabolic rate. Instead we tested the effect of sine and cosine of month. The final model for sea lions included water temperature and mass as fixed effects with individual as the random effect. Animal ID influenced the model as the variance explained was improved (LME: AICc = -157.6, logLik = 83.8, R² (conditional) = 0.778, R²(marginal) = 0.827). Neither, sine month, cosine month, moult, sex or age, contributed to the final model. Sea lions housed at RF1 and RF3 were exposed to a wide range of water temperatures (16-27°C) and there was a negative relationship between sSMR and water temperature (Fig. 2.6A-D). Sea lions from RF2 were measured in water temperatures of 22-27°C but there was no relationship between sSMR and water temperature.

Table 2.2 Monthly mass-specific standard metabolic rates (sSMR) of six fur seals. Mean \pm SE and N of mass-specific standard metabolic rate (sSMR ml O₂ min⁻¹ kg⁻¹) and multiple of Kleiber's (1975) predicted basal metabolic rate (BMR*) for an Australian fur seal female (AFF), male (AFM) and three New Zealand fur seal males (NFM), measured in different months. (The number following the species and sex id is an individual identifier). **Bold** values indicate months when the seal was moulting. NA indicates a month when that individual was not measured.

Month	Measure	Feb	Mar	May	Jul	Aug	Sep	Oct	Nov	Dec
Australian fur seal										
AFF1	sSMR	7.0±0.2	6.1±0.5	5		6.3±0.6	5.0		6.7	8.2±0.5
	BMR*	2.1	1.8	NA	NA	1.9	1.5	NA	1.5	2.0
	Ν	3	4			2	1		1	2
AFM1	sSMR	6.7 ± 0.5	5.5±0.4	ł		3.6±0.8	3.1		4.9	6.1 ± 0.5
	BMR*	2.6	2.1	NA	NA	1.4	1.2	NA	1.8	2.3
	Ν	3	7			2	1		1	2
New Ze	ealand fur s	seal								
NFM1	sSMR	6.5±0.3	6.1±0.2	2		4.0±0.3			5.4	4.3±0.00
	BMR*	2.3	2.0	NA	NA	1.4	NA	NA	2.0	1.5
	Ν	3	6			3			1	2
NFM2	sSMR			5.8 ± 0.2					5.2±0.1	
	BMR*	NA	NA	1.9	NA	NA	NA	NA	1.9	NA
	Ν			2					2	
NFM3	sSMR	8.2±0.02	$29.8\pm0.48.8\pm0.5$		5.9±0.2 6.7				7.5 ± 0.2	
	BMR*	2.5	2.7	2.4	1.7	1.9	NA	NA	2.1	NA
	Ν	3	2	2	2	1			2	


Figure 2.5 Boxplots of Australian sea lion sSMR by month and sex. Median, interquartile range (box) and range (bars) of mass-specific standard metabolic rate (sSMR ml $O_2 min^{-1} kg^{-1}$) for male (grey box, N = 2) and female (white box, N = 5) Australian sea lions over the course of the year.



Figure 2.6 Relationship between sSMR (ml O₂ min⁻¹ kg⁻¹) and water temperature ($W_T^{\circ}C$) for four Australian sea lions. A) Adult male (ASM1 - sSMR = 9.79 – 0.16 × WT, logLik = -12.40, R2 = 0.450, p = 0.017, n = 12). B) Adult female (ASF4 - sSMR = 9.26 – 0.12 × WT, logLik = -13.16, R2 = 0.348, p = 0.034, n = 13). C) Adult female (ASF2 - sSMR = 12.67 – 0.20 × WT, logLik = -14.38, R2 = 0.497, p = 0.011, n = 12). D) Adult male (ASM2 - sSMR = 7.96 – 0.12 × WT, logLik = -11.73, R2 = 0.336, p = 0.038, n = 13).

Table 2.3 Monthly mass-specific standard metabolic rates (sSMR) of six Australian sea lions. Mean \pm SE and N of mass-specific standard metabolic rate (sSMR ml O₂ min⁻¹ kg⁻¹) and multiple of Kleiber's (1975) predicted basal metabolic rate (BMR*) for five Australian sea lion females (ASF) and two Australian sea lion males (ASM) measured in different months. (The number following the species and sex id is an individual identifier). **Bold** values indicate months when the seal was moulting. NA indicates a month when that individual was not measured.

Month	Measure	Feb	Mar	May	Jul	Aug	Sep	Oct	Nov	Dec
Australian sea lion										
ASF1	sSMR	8.1±0.5	57.2±0.4	9.2	9.7±0.4			8.8	7.1	
	BMR*	2.2	1.9	2.4	2.6	NA	NA	2.4	1.9	NA
	Ν	3	3	1	3			1	1	
ASF2	2 sSMR 7.9±0.4 7.8±0.4			7.0±0.9 7.6				7.1	7.9 ± 0.1	
	BMR*	2.2	2.2	NA	NA	1.9	2.1	NA	2.0	2.3
	Ν	3	6			2	1		1	2
ASF3	sSMR	7.6±0.6 6.3±0.3				7.0±0.6			7.2	7.6 ± 0.4
	BMR*	2.3	1.9	NA	NA	2.1	NA	NA		2.2
	Ν	3	6			3			1	2
ASF4	sSMR	6.7±0.1	5.9±0.3	7.5±0.8	37.5 ± 0.3			6.4	5.7	
	BMR*	2.2	2.2	2.0	2.1	NA	NA	2.0	2.3	NA
	Ν	3	3	2	3			1	1	
ASF5	sSMR	R 9.3±0.3 8.1±0.3			6.4±0.9			6.3	7.6 ± 0.2	
	BMR*	2.8	2.4	NA	NA	1.9	NA	NA	1.9	2.3
	Ν	2	5			3			1	2
ASM1	sSMR	5.9±0.3	35.6±0.4	6.5±0.2	27.5±0.3			8.2	7.2	
	BMR*	2.1	1.9	2.2	2.5	NA	NA	2.7	2.4	NA
	Ν	3	2	2	3			1	1	
ASM2	sSMR	4.9±0.3 4.2 6.0±0.2		6.0 5.8±0.4				5.1±0.0	5	
	BMR*	1.8	1.6	2.2	2.2	2.2	NA	NA	1.9	NA
	Ν	5	1	2	1	2			2	

2.4. Discussion

2.4.1. Standard metabolic rate

Measuring animals in captivity provides an excellent proxy for estimating the energy expenditure of wild populations. Respirometry is considered the 'gold-standard' method of measuring metabolic rate, but is inherently difficult to use in the wild (Boyd 2002, Halsey *et al.* 2009a, Dalton *et al.* 2014b). Therefore, these types of experiments allow us to develop our understanding of animal physiology, with minimal impact on populations, and utilising the most accurate technology available. We measured the SMR of

three species of otariid (N=12) at rest in water during significant times of their annual cycle. We found that the mean sSMR for the seals in this study were 2 to 2.2 times (Table 2.1) that predicted by Kleiber (Kleiber 1975), which falls within the range predicted for a marine mammal (Williams *et al.* 2001), and is lower than the in-air resting metabolic rate of juvenile northern fur seals (2.9 times predicted; Dalton *et al.* 2015) and than the average daily metabolic rate of lactating northern fur seals (3.2 times predicted; Costa and Gentry 1986).

Few studies have successfully measured true basal metabolic rate of pinnipeds under the strict conditions of Kleiber (1975). Our animals were adult, non-reproductive, quiescent and post-absorptive, but they were measured for a relatively short time, in water and we could not be certain they were in their thermoneutral zone (TNZ – the temperature range at which the body does not need to work harder to warm up or cool down). We measured seals in the morning before they became active, and only if they were dry, suggesting they had been resting and not swimming prior to measurement. We measured them in water as they were habituated to this experimental set-up, and were noticeably calm during experiments, corresponding to their relatively low metabolic rate, despite not meeting all the conditions of Kleiber (1975). The range of average sSMR for the animals in this study was 5.3 to 7.4 ml O₂ min⁻¹ kg⁻¹; (Dassis *et al.* 2012). In that study, a single captive animal had resting metabolic rate within the range of the wild animals that were measured simultaneously. We therefore consider our results to be a good estimate of resting metabolic rate in these species and our measurement of sSMR is probably approaching basal metabolic rate.

2.4.2. Influence of the annual cycle on metabolic rates

The stages of the annual cycle that are energetically costly are the preparation for and the recovery from annual breeding, including the annual moult. Thus, it is expected that the moult and breeding will have the greatest influence on the variation in the metabolic rate of pinnipeds (Costa and Trillmich 1988, Rosen and Renouf 1995). Australian sea lions have different reproductive and moulting strategies to every other otariid, while the Australian fur seal and New Zealand fur seal have typical yearly cycles of pinnipeds. Australian fur seals and New Zealand fur seals are similar, with breeding and pupping occurring during the Austral summer, followed by a moult (Goldsworthy and Shaughnessy 1994, Gibbens and Arnould 2009). In contrast, Australian sea lions have an asynchronous breeding and moulting cycle, where females come into oestrus every 17-18 months and moulting can occur year round for three to four months (Higgins 1993, Gales *et al.* 1994). This lack of synchronization was evident in the sSMR of the sea lions as there were no significant changes over the course of the year, whereas fur seals had distinct changes in their sSMR coinciding with the moult and the build-up of body condition prior to the breeding season.

2.4.2.1. Fur seals

In the preparation for and recovery from breeding, male and female fur seals have different motivations for fat accumulation, though their physiological responses appear similar. Females allocate their energy resources to fat stores for gestation and milk production that must be maintained year round if the female is pregnant or lactating (Costa 1991a). Females are usually pregnant during the pre-moult period and must be in good condition to birth and feed a new pup (Boyd *et al.* 1995a). Females in better condition more often give birth to larger pups that have a higher chance of survival (Guinet *et al.* 1998). Dominant males require a large body mass during the breeding season to successfully establish and defend territory and reproduce, while during the non-breeding season they generally maintain a lower body mass when they leave the breeding areas to forage (Boyd and Duck 1991, Carey 1991). This is an energetically costly endeavour that only large, healthy males can achieve (Boyd and Duck 1991). We found that the rate of energy expenditure from male and female fur seals was consistent with that expected for wild fur seals (Costa and Gentry 1986). Male sSMR was highest during the moult and lowest during the post-moult, after which it increased again before the breeding season. In females resting metabolic rate was much more consistent, increasing from the post-moult (lowest) to the pre-moult (highest). These metabolic changes align with body conditions of wild Australian fur seals, where females blubber distribution does not change over the course of the year, while males undergo large seasonal shifts (Carey 1991, Arnould and Warneke 2002).

We found that for both fur seal species, sSMR was the lowest during the post-moult (Austral autumn and winter) period. The reduction in sSMR during this period is likely a strategy to maintain body condition during periods of reduced prey availability and increased thermoregulatory demands. For both fur seal species, the post-moult period corresponds to the lowest sea-surface temperatures, and presumably the time of lowest productivity within their home ranges (Harris *et al.* 1991, Baylis *et al.* 2008b). Lactating Australian and New Zealand fur seals tend to undertake their longest foraging trips (Arnould and Hindell 2001a, Harcourt *et al.* 2002, Page *et al.* 2005b, Baylis *et al.* 2008a, Kirkwood and Arnould 2011) and seals tend to maintain a lower body mass (corresponding to a low metabolic rate) following the moulting and breeding season (Arnould and Warneke 2002, Beck *et al.* 2003a, Sparling *et al.* 2006).

Sometime before the breeding season male fur seals increase their metabolic rate from the post-moult period (Fig. 2.3). The female Australian fur seal also showed an increase, although not as pronounced as for males. It may be important that this event is synchronised for males and females such that they reach sexual maturity simultaneously each year (Boyd 1991). The timing also corresponds to the accumulation of fat, as the seals get fatter, their metabolic rate increases (Beck *et al.* 2003a). As we were unable to measure the seals year round the exact timing of this phenomenon is unknown, although it is likely consistent with the onset of spermatogenesis for males, which begins 3-4 months prior to breeding season (Stewardson *et al.* 1998, Stewardson 2007). Spermatogenesis is energetically expensive, particularly for large mammals (Gomendio *et al.* 2011).

Males and females have different energy requirements at different times that can be achieved by either eating more or reducing energy use. Our results demonstrate that seals decrease their energy use during times of fat accumulation and anecdotal evidence from captivity suggests that the quantity of food increases during this time for fur seals (pers. comm. A. Tolley, M. Ryan, R. Tate). In the wild, New Zealand fur seals target higher energy prey close to the breeding season (Page *et al.* 2005a), and Australian

fur seals make longer foraging trips (Arnould and Hindell 2001a), but neither increase their foraging effort (Kirkwood *et al.* 2006). Therefore, it is possible that to aid fat accumulation without an increase in foraging effort, fur seals depress their metabolism and possibly encountering more prey items, as a result of an increase in prey availability.

2.4.2.2. Sea Lions

Australian sea lions show little variation in metabolic rate over the year, as demonstrated by the lack of significance of month in the overall model. This is consistent with results of Williams *et al.* (2007) who found that resting metabolic rate of Californian sea lions (*Zalophus californianus*) showed little change across seasons. Sustaining a consistent sSMR may be a strategy for sea lions to maintain their asynchronous breeding cycle. The lack of seasonal variation in the metabolic rates of Australian sea lions are reflected in their temporally stable and geographically fixed foraging patterns (Lowther *et al.* 2011, Lowther *et al.* 2013). Despite substantial individual variation in foraging strategies, Australian sea lions forage at the same trophic level in the same regions over seasons and years (Lowther *et al.* 2011, Lowther *et al.* 2013). There were no seasonal changes in metabolic rates observed (this study) or foraging strategies (Lowther *et al.* 2011) and the availability of sea lion prey is consistent, even if low, year round (McIntosh *et al.* 2006, Peters *et al.* 2015). This means that Australian sea lions are likely to adopt other behaviour strategies, such as increasing their food intake, to cope with additional energetic costs throughout the year (e.g. lactation; Williams *et al.* 2007).

As male Australian sea lions are not able to use seasonal cues in their environment to predict the onset of the breeding cycle, we contend that they maintain a constant sSMR and a static foraging strategy, remaining close to the breeding colonies to be prepared for breeding with females at any time of year (Lowther *et al.* 2013, Ahonen *et al.* 2016). This is likely an adaption to a low productivity environment that is fairly constant (McKenzie *et al.* 2005, Villegas-Amtmann *et al.* 2009). Australian sea lions breeding period lasts for 120 days, suggesting that males must have an extended period of spermatogenesis (Ahonen *et al.* 2016). Males conserve energy by "mate-guarding" – choosing a single female to mate with from when they haul out until they go into oestrus (Higgins 1990). Following mating, they leave to forage or to mate at another near-by colony, and therefore may not have the option of layering additional blubber prior to the next period when spermatogenesis and mate guarding occur (Ahonen *et al.* 2016).

2.4.3. Temperature

We show that some of the variation in metabolic rates of fur seals and sea lions can be explained by changes in natural fluctuations in water temperature within each facility (Figs. 2.5 and 2.6). Though we did not measure the sSMR of fur seals or sea lions in water below 16°C, nor do we know if the seals are within their thermoneutral zone (TNZ), there appears to be an increase of sSMR with increasing water temperature for fur seals (Fig. 2.5A-D) and a decrease in sSMR with increasing water temperature for sea lions (Figs. 2.6A-D). Sea lions who were housed at the highest latitude (RF3) did not demonstrate variations in sSMR from 22°C to 26°C, suggesting they were within their TNZ. The different responses to temperature are likely due to differences in thermoregulatory strategies. Fur seals rely on a thick layer of fur to thermoregulate, as the blubber layer they maintain is metabolically inert and used primarily for

energy storage (Liwanag *et al.* 2012a, Dalton *et al.* 2014a). Sea lions rely on a thicker blubber layer to protect themselves from cold water (Mellish *et al.* 2007, Williams *et al.* 2007, Liwanag *et al.* 2012b) that is interspersed with layers of muscle (Mellish *et al.* 2004). It is possible that sea lions metabolic rate declines during warmer temperatures as they utilise their metabolically active blubber layer through blood perfusion – dilating blood vessels to allow blood to flow through and be warmed by the outside temperature (Meagher *et al.* 2008, Liwanag *et al.* 2009) – thus reducing the metabolic costs of maintaining a constant body temperature.

Maintenance of these thermoregulatory strategies is correspondingly different in the two families, each with its own energetic cost. Fur seals use a layer of air trapped between their fur layers to insulate their body. This allows the skin to be maintained at body temperature, but requires that fur seals spend a significant amount of time grooming their pelage (Battaile *et al.* 2015). This is an energetically expensive tactic (Liwanag 2010), but could be complementary in cold water as it would raise metabolic rate. At warm temperatures fur seals increase their metabolic rate in order to encourage blood flow to the flippers that are unprotected by hair to cool down (Dalton *et al.* 2014a), while in cool temperatures fur seals restrict blood flow to these areas in order to minimise heat loss (Mostman-Liwanag 2008). As sea lions rely solely on their blubber to remain warm, they must retain a thicker layer than fur seals to compensate (Scholander *et al.*, 1950), which can only be maintained by consuming large amounts of energy. Sea lion blubber thickness appears to remain constant throughout the year (Mellish *et al.*, 2007), which may be why the metabolic rate of sea lions relatively constant across months, but declines when water temperature increases. Despite the substantial differences in the thermoregulatory strategies of fur seals and sea lions, there was little difference in their overall sSMR, suggesting that these strategies have complementary costs.

2.4.4. Sex

A significant effect of sex on sSMR was found for both sea lions and fur seals where females had higher mass-specific metabolic rates than males. This same effect has been found in other species of adult pinniped including; Californian sea lions (Hurley and Costa 2001); grey seals (Beck *et al.* 2003a) and Antarctic fur seals (*A. gazella*) (Boyd and Duck 1991, Boyd and Croxall 1996). Pinniped juveniles and pups do not show any significant sex differences in their metabolic rates, instead maintaining a consistently elevated metabolic rate associated with the cost of growth (Fowler *et al.* 2007b, Verrier *et al.* 2011). As they age morphological and physiological differences arise including extreme sexual dimorphism and an elevated mass-specific metabolic rate in the female (Hurley and Costa, 2001), that does not change depending on reproductive status (Williams *et al.* 2007). Females are usually in a stage of reproduction throughout the year (lactating or pregnant), while males spend some of the year removed from reproductive constraints. By measuring females that were non-breeding and non-lactating we removed the effect of reproduction yet females still had elevated sSMR in comparison to the males. Therefore, the higher sSMR that we observed was probably related to the ongoing costs of reproduction. As there is no evidence that the metabolic rate of otariids varies between reproductive and non-

reproductive cycles (Costa and Gentry 1986, Williams *et al.* 2007), these differences in sSMR are likely due to allometry (Kleiber 1975, McNab 2008).

2.4.5. Moult

In pinnipeds, the moult occurs following the breeding period, either immediately after breeding or following a brief foraging period (Scheffer and Johnson 1963). For Australian and New Zealand fur seals the moult occurs early in the year for around two months. Australian sea lions can moult at any time of year and the moult is generally extended over 3-5 months. Metabolic responses to this phenomenon differ across species, and the energetic processes behind the moult are not well understood. In our study the male fur seals increased sSMR during the moult but there was no consistent effect of moult on the sSMR for any of the other seals. Harbour, spotted (P. largha) and northern elephant seals (Mirounga angustirostris) have low resting metabolic rates during their moult (Ashwell-Erickson et al. 1986, Worthy et al. 1992). Northern fur seals (Callorhinus ursinus) metabolic rate was highest during autumn and lowest in the winter, which corresponded to the beginning and the end of the moult (Dalton et al. 2015). Grey seals and non-reproductive Californian sea lion females showed increased metabolic rate during the moult, juveniles significantly more than adults (Boily 1996, Boily and Lavigne 1997, Beck et al. 2003a, Williams et al. 2007). Increasing metabolic rate during the moult is proposed to aid in thermoregulation for fur seals while some of the insulating layer is lost and from the energy invested into the growth of new hair (Boyd et al. 1993). Decreasing metabolic rate is proposed to delay fat loss while hauled-out during the moult (Beck et al. 2003a).

As sea lions do not rely on their fur layer for thermoregulation, their energetic response to the moult is likely to differ from the fur seals. During the moult the blubber layer and lipid content of Californian sea lions is at its lowest, suggesting that an increase in metabolism is required to maintain body temperature within the thermoregulatory range (Williams *et al.* 2007). As the seals in our study were housed in warm water for the duration of their moult, the effect of the moult may have been masked. The female sea lions displayed no discernible pattern in sSMR during the moult (Table 2.3). The two male sea lions in this study moulted at different times of the year, one during the warmest water period (25-26°C), when we recorded his highest sSMR (ASM1 - Table 2.3) and the other during moderate water temperatures (19-20°C) when we recorded his lowest sSMR (ASM2). If the sea lions do indeed use perfusion to cool during warm temperatures, this effect may have been exacerbated by the moult allowing the body to cool quicker and slowing their metabolism. During the period of moderate water temperatures, the sea lions may need to increase their metabolic rate to cope with the cooler water and hair loss. Seal moult generally occurs in summer to maximise skin surface temperature for the promotion of hair growth (Paterson *et al.* 2012) and since they are unable to efficiently thermoregulate (Feltz and Fay 1966).

2.4.6. Implications for a changing environment

Australian sea lions typically forage in temperatures of 12 to 22°C in South Australia (Lowther *et al.* 2013). Male New Zealand fur seals forage in waters around Macquarie Island (54°S, 159°E) where the water temperature can be as low as 2°C to Montague Island (36°S, 150°E) and across to western Australia

(33°S, 114°E), where the water can reach 24°C (Campbell *et al.* 2014, McIntosh *et al.* 2014). Australian fur seals are found predominantly in the Bass Strait, southern Australia, where water temperatures have a much smaller range of 12.6 to 19.3°C (Kirkwood *et al.* 2006, McIntosh *et al.* 2014). Therefore, the fur seals and sea lions in our study were exposed to a range of temperatures that were at the upper limit of what they would experience in the wild. Despite prolonged exposure to water temperatures higher than those that seals would experience in the wild, metabolic rates were not outside those expected for a marine mammal (Williams *et al.* 2000), suggesting that the otariids were within their TNZ. It is possible therefore, that the fur seals and sea lions housed in captivity have acclimatised to warm water. Southeast Australia is expected to have some of the largest increase in SST globally with 0.7-1.4° warming by 2030 (Ridgway and Hill 2012, Carroll *et al.* 2016), and this study presents evidence that the fur seals and sea lions that occupy this area have the physiological capacity to adapt to these changes.

2.4.7. Conclusion

Animals that have a limited ability to adjust their energy storage and usage may be more susceptible to environmental change. Maximising fitness can, in part, be achieved through adjusting metabolic rates in response to changes in environmental conditions. Flexibility in physiological and morphological traits are important to survival as mammals that have static metabolic rates and core body temperatures are more likely to be extinct (Geiser and Turbill 2009). Australian fur seals and New Zealand fur seals demonstrated annual variations in their standard metabolic rates, which corresponded to their annual breeding and moulting cycle. Australian sea lions showed very little variation in metabolic rate over the year or in response to the moult, but metabolic rate reduced in response to increasing water temperatures. Animals in this study have demonstrated adaptations to warming water, a trait that may enhance their survival in a changing environment. Fur seal numbers in Australia are increasing, while sea lions are in decline and classified as endangered. Sea lions may compensate for living in a low productivity environment by utilising an 18-month breeding cycle and a static foraging strategy and energy usage (Lowther and Goldsworthy 2011, Ahonen et al. 2016). Further environmental change may mean that they are ready to withstand further change, or they may already be on the precipice of their demise. In contrast, fur seals may be buffered by their potential to utilise their pelagic diving abilities to move offshore and exploit cold upwellings.

Chapter 3

Swimming metabolic rates vary by sex and development stage, but not by species, in three species of Australian otariid seals

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Abstract

Physiology may limit the ability for marine mammals to adapt to changing environments. Duration of foraging dives are a function of total available oxygen stores, which theoretically increase as animals grow, and metabolic costs. To evaluate how physiology may influence the traveling costs for seals to foraging patches in the wild, we measured metabolic rates of a cross-section of New Zealand fur seals, Australian fur seals and Australian sea lions representing different foraging strategies, development stages, sexes and sizes. We report values for standard metabolic rate, active metabolic rate (obtained from submerged swimming), along with estimates of cost of transport (COT), measured via respirometry. We found a decline in mass-specific metabolic rate with increased duration of submerged swimming. For most seals mass-specific metabolic rate increased with speed and for all seals mass-specific COT decreased with speed. Mass-specific metabolic rate was higher for subadult than adult fur seals and sea lions, corresponding to an overall higher minimum COT. Some sex differences were also apparent, such that female Australian fur seals and Australian sea lions had higher mass-specific metabolic rates than males. There were no species differences in standard or active metabolic rates for adult males or females. The seals in our study appear to operate at their physiological optimum during submerged swimming. However, the higher metabolic rates of young and female fur seals and sea lions may limit their scope for increasing foraging effort during times of resource limitation.

Key words

Energetics, otariid, foraging strategy, cost of transport

3.1. Introduction

Like all air-breathing marine mammals, pinnipeds find their food through diving at sea, and their diving abilities are influenced by physiological, morphological and behavioural characteristics (Kooyman 1989, Ponganis 2015). Pinnipeds actively hunt prey that is patchily distributed throughout the water column, often found at great depths or at great distances from where they haul out (Staniland *et al.* 2007). The distance that they can travel and the depths they reach are a function of their total available oxygen stores, which theoretically increase as animals grow, and of their overall metabolic costs (Kooyman and Ponganis 1998). An animal's physiological capabilities may be influenced by intrinsic factors such as sex, developmental stage and species (Fowler *et al.* 2007b, Weise and Costa 2007, Ochoa-Acuña *et al.* 2009), and extrinsic factors such as swimming speed, dive depth and duration and distance travelled (Williams 1999, Hastie *et al.* 2006, Staniland *et al.* 2007). Understanding these relationships is essential for developing precise energy budgets. By measuring metabolic rates from pinnipeds at different development stages, and comparing across taxa, we can begin to understand the diversity of energetic requirements, and can also quantify physiological constraints on their ability to find and obtain food (Costa *et al.* 1989, Hastie *et al.* 2007).

The primary energetic costs above basal metabolic rate for marine mammals, after growth and reproduction, derive from movement, through activities such as travelling to a foraging patch, diving, hunting and chasing and capturing prey (Karasov 1992, Williams *et al.* 2015). By determining the cost of transport (COT), defined as the energy expended in moving one unit of body mass over one unit of distance and usually measured in J kg⁻¹m⁻¹ (Schmidt-Nielsen 1972), it is possible to make intra- and inter-specific species comparisons (Williams 1999, Fish 2000). COT varies depending upon the method of propulsion used and positioning in the water column (Rosen and Trites 2002) and likely plays a role in prey choice, as animals must positively balance total energy intake over the cost expended in travelling a given distance in order to get that energy (Staniland *et al.* 2007). For example, Antarctic fur seals (*Arctocephalus gazella*) that have travelled longer distances to foraging areas generally take higher energy prey (Staniland *et al.* 2007), as seals on longer trips have a higher overall metabolic rate compared to seals on shorter trips from the same colony (Arnould *et al.* 1996).

Overall diving abilities in pinnipeds vary across life stages. Like many growing animals, young marine mammals have disproportionately higher metabolic rates (energetic costs) than adults (Fowler *et al.* 2007b, Verrier *et al.* 2011). They also have lower oxygen stores, largely due to their smaller size, but partly due to their relatively lower iron intake and development stage (Horning and Trillmich 1997a, Burns *et al.* 2004, Weise and Costa 2007). The development of adult metabolic rates and oxygen stores appears related to the dependency period of pups (Burns *et al.* 2004). In phocids, oxygen storage capacity and metabolic rate develop to almost adult levels soon after independence, most likely a reflection of shorter nursing time and early weaning period (Costa 1991a, Costa 1993, Burns *et al.*

2004). Otariid pups have a longer period of dependency than phocids, (Costa 1991a, Costa 1993) and in some species do not appear to develop their oxygen storage capacity fully until they become adults (e.g. Fowler *et al.* 2007b, Weise and Costa 2007). Within the otariid group there appears to be variation beyond that which corresponds to weaning period in the rate of development of oxygen carrying capacity. For example, Steller sea lions (*Eumetopias jubatus*) attain near adult capacity as juveniles (~29 months; Richmond *et al.* 2006), contrasting with the Californian sea lions (*Zalophus californianus*), which may take up to six years (Weise and Costa 2007).

Similarly, strategic allocation of energy to meet needs for reproduction differs for male and female pinnipeds and therefore may contribute to differences in metabolic rates. Females are generally pregnant or lactating throughout the year, requiring a minimum threshold of energy stores (Harcourt et al. 2001). In males, body size fluctuates necessarily, being larger during the breeding season in order to compete with other males for territory and females, and to be able to draw on fat reserves while hauled out (Boyd and Duck 1991). Australian sea lion males have a unique strategy, intermittently feeding throughout the breeding period and mating with a single female at a time, a likely consequence of their extended breeding period (Higgins and Gass 1993, Ahonen et al. 2016). The physiological effects of these roles across species remains unclear. There is evidence that some female otariids (Steller sea lions and Californian sea lions) have higher total oxygen stores relative to mass than males (Richmond et al. 2006, Weise and Costa 2007). Mass-specific metabolic rates also differ such that Californian sea lions (Hurley and Costa 2001) and harbour seal (Phoca vitulina) (Rosen and Renouf 1995) females have higher rates, whereas in Southern sea lions (Otaria flavescens) subadult males metabolic rates are not significantly different from adult females (Dassis et al. 2012). South American sea lion (Otaria byronia) juvenile males have larger mass-specific total oxygen stores than females, but habitat characteristics are better able to explain their diving capabilities (Hückstädt et al. 2016).

There are three pinniped species native to Australian coastal waters: the Australian fur seal (*Arctocephalus pusillus doriferus*), the New Zealand fur seal (*Arctocephalus forsteri*) and the Australian sea lion (*Neophoca cinerea*). The three species have markedly different foraging strategies and prey preferences in their overlapping habitats, perhaps in part due to differing energetic potentials (Costa *et al.* 2004). Both the Australian sea lion and the Australian fur seal employ a benthic foraging strategy concentrating on the continental shelf, with sea lions predominantly consuming fish, cephalopods and crustaceans (McIntosh *et al.* 2006), while fur seals target fish and cephalopods (Page *et al.* 2005a, Littnan and Arnould 2007). The New Zealand fur seal is an opportunistic forager, concentrating on vertically migrating fish and cephalopods in the pelagic zone (Harcourt *et al.* 2002, Page *et al.* 2005a). The foraging dives of the Australian fur seal and sea lion are generally greater in duration than the New Zealand fur seals, presumably because of a larger body size and potentially greater oxygen stores and lower metabolic rates (Arnould and Hindell 2001a, Mori 2002, Page *et al.* 2005b, Fowler *et al.* 2007a, Fowler *et al.* 2007b).

It has been hypothesised that benthic divers are operating closer to their physiological capacity than pelagic divers (Costa and Gales 2000, Fowler *et al.* 2007b) and therefore may not have the flexibility to adapt to a changing environment (Costa *et al.* 2004, Arnould and Costa 2006). The southern Australian marine ecosystem in which these species reside is rapidly changing with varied rates of ocean warming, potentially influencing prey abundance and distribution (Cai *et al.* 2005). Presumably, the foraging strategies that these species evolved were to meet the demands of different environmental pressures than are being experienced now, bringing uncertainty to which species, if any, will cope. This study aims to investigate the inter- and intra-specific physiological capabilities of a comparative group of seals with different foraging strategies and life history traits, in order to identify the flexibility they might have to changes in the distribution of prey resulting from environmental changes.

3.2. Materials and Methods

3.2.1. Animals

We conducted metabolic experiments at three Australian marine facilities from March to May (austral autumn) and October to December (austral spring) 2014 using three New Zealand fur seals, two Australian fur seals and seven Australian sea lions. Three sea lions were from Dolphin Marine Magic, Coffs Harbour (RF1: 30°17'S, 153°8'E; animal codes: ASF1, ASF3, ASM1); three fur seals and three sea lions were from Underwater World, Sunshine Coast (RF2: 25°40'S, 153°7'E; animal codes: NFM3, AFM1, AFF1, ASF2, ASF4, ASF5); and two fur seals and one sea lion were from Taronga Zoo, Sydney (RF3: 33°50'S, 151°14'E; animal codes: ASM2, NFM1, NFM2) . All animals were non-reproductive during the study period, were on permanent display at their respective facilities, and were cared for under the husbandry guidelines of the individual facility. The study was approved by Macquarie University ethics committee (ARA-2012_064) and Taronga ethics committee (4c/10/13). All Australian sea lions in this study were born as a part of an ongoing captive breeding program in Australian aquaria, whereas all fur seals came into captivity as juveniles having been found in poor health or injured and were considered unsuitable for release back into the wild after rehabilitation. Fur seal ages were estimated from their size and condition when they were introduced to their facility. Seals ranged in age from 5 to 24 years, and mass from 45 to 177kg (Table 3.1).

3.2.2. Experimental protocol

We measured standard metabolic rate (SMR) and active metabolic rate (AMR) using open-flow respirometry. Prior to testing, animals were trained for a minimum of six months to swim submerged for increasing durations (with the aim of achieving 3 minutes duration), and to surface in a clear moulded acrylic hood (80L), that floated on the surface of the water and was connected to a field metabolic system (FMS: Sable Systems International, Inc., Henderson, NV, USA). To adhere to the

conditions of Kleiber (1975) seals were not fed for a minimum of 14 hours (post-absorptive), were resting in husbandry pools prior to trials, were adult (9 of 12 seals), were not pregnant and remained within their assumed thermo-neutral zone during the trials (as determined by water temperature – (Ladds *et al.* 2017a)). Water temperature for this study ranged from 18 to 26° C (mean 22.3 ± 3.1). Seals were given free choice to complete the tasks. When seals surfaced within the hood they were rewarded with a small amount of food, but this was not expected to influence the metabolic rates of the seals over the short duration of the trial (Rosen and Trites 1997, Rosen *et al.* 2015). Trials ran for no more than 30 minutes per seal. Mass (± 2 kg) of each individual was measured once per week during the trials.

To estimate SMR seals would float under the hood for a minimum of three minutes. The most stable 120 seconds of data was used to estimate SMR. To estimate AMR seals would swim submerged for a pre-determined distance or time before returning to the hood where they remained until their metabolic rate returned within 5% of baseline levels, which took between 2 and 6 minutes. Swimming distance and submerged duration differed due to differences in pool dimensions at each facility, training techniques, and motivation of individual seals on the day of the trial. Trials where seals surfaced outside of the hood were excluded from the analysis. We recorded trials with a Sony HDRSR11E camcorder (Sony, Japan) (above water) and a GoPro HERO3 (GoPro, USA) (below water), and used the video footage to calculate the distance travelled underwater and duration of swimming to determine speed. Distance travelled was estimated by measuring the length between the turning points for each seal in their respective pool and multiplying this by the number of lengths swum. Swim durations were also timed *in situ.*

The hood was connected to an open-flow respirometry system (Sable Systems International, Inc., Henderson, NV, USA) where air was pulled from the hood with a Sable Systems Mass Flow pump at an adjustable flow rate ranging from 300-450 l min⁻¹. We adjusted the flow for each individual to ensure that the oxygen inside the hood remained above 20%. A continuous sub-sample was drawn into the analyser at 200 ml min⁻¹ and measured for water vapour concentration, then dried using magnesium perchlorate and scrubbed of carbon dioxide with soda lime before entering an FC-1 oxygen analyser. To ensure that the measured CO₂ and water vapour fluctuations did not exceed 1% and 5% respectively, the scrubbers were monitored using the built-in CO₂ analyser and an external water vapour analyser. The percentage of oxygen in the expired air was measured continuously with Sable Systems ExpeData software and recorded at 5 samples per second. Oxygen consumption ($\dot{V}O_2$) was calculated using equations from Withers (1977) assuming a respiratory quotient of 0.77 (Feldkamp 1987, Boyd *et al.* 1995b):

$$\dot{V}O_2 = \frac{\dot{V_E} \cdot (F_{IO_2} - F'_{EO_2})}{1 - F_{IO_2} + RQ \cdot (F_{IO_2} - F'_{EO_2})}$$
(3.1)

where $\dot{V_E}$ = excurrent flow rate; F_{IO_2} = incurrent fractional oxygen concentration; F'_{EO_2} = excurrent fractional oxygen concentration (CO₂ removed); RQ = estimated respiratory quotient.

We calibrated the system every two to three days using nitrogen (N₂) and ambient air following the method of Fedak *et al.* (1981). Nitrogen gas (99.99%) was passed through a flow meter (Sable Systems FoxBox) at a known rate. The observed values were within 5% of predicted N₂ values.

3.2.3. Active metabolic rate

To calculate the amount of energy used during activity above basal costs, the pre-dive oxygen consumption multiplied by the swim time was subtracted from the post-dive oxygen consumption divided by the total recovery time.

$$AMR = \frac{VO_{2 \text{ post dive}} - VO_{2 \text{ pre dive}} \times recovery time}{swim duration}$$
(3.2)

where AMR = active metabolic rate, $VO_{2 post dive}$ = oxygen consumption measured after activity, $VO_{2 pre dive}$ = oxygen consumption measured before activity.

3.2.4. Cost of transport

Cost of transport was defined as the energetic cost of transporting one unit of body weight over one unit of distance (Schmidt-Nielsen 1972). This can be reported as COT_{NET} ; the net cost of transport, which takes into account only the movement of body and limbs, or COT_{TOT} ; the total cost of transport, which is the cost of moving the body plus the maintenance costs (Williams 1999). In this study, we used COT_{NET} (henceforth COT) that we calculated by dividing total mass-specific active metabolic rate (calculated above) for the dive by speed.

$$COT_{NET} = \frac{sAMR(W/kg)}{speed (m/s)}$$
(3.3)

where sAMR = mass-specific active metabolic rate

3.2.5. Statistical analysis

We used linear mixed-effects models (LMM) to examine the variation in sSMR and sAMR of the eight classes of seal. Seals were grouped into classes based on their species, sex and development stage (i.e. adult or subadult). Subadult animals were classed as such based on their presence or absence of a mane in males and size in females (Gales *et al.* 1994). One Australian sea lion and one New Zealand fur seal male were classed as subadult as they had not reached the size or developed a mane typical of adults. One Australian sea lion female was classed as subadult as she had not reached typical adult mass or length. We used the R package *nlme* (Pinheiro *et al.* 2014) to run the LMM. With sSMR as the response variable we used class as the fixed effect and season as a covariate. With sAMR as

and location were also tested as covariates but were not significant in the model and were therefore removed. We used animal ID to account for repeated measures effects.

We used simple linear regressions to investigate the relationship between swim duration and sAMR. Non-linear least-squares regression were used to examine the relationship between COT and speed, and sAMR and speed, for each of the eight classes of seal. All analyses were run in R, version 3.2.3 (R Core Development Team 2015). We report values as means (\pm SD) and significance was set at $\alpha = 0.05$.

3.3. Results

3.3.1. Standard metabolic rate

sSMR was measured on all days prior to beginning trials and served as the baseline measure to which the seals would return after swimming. Mean sSMR ranged from 8.84 to 15.09 ml O₂ min⁻¹ kg⁻¹, which is 3.0 to 3.9 times the predicted BMR for a terrestrial mammal of equal size (Kleiber 1975). There were some significant intra- and inter-species differences in sSMR. sSMR for the two adult male New Zealand fur seals was 9.42 ± 1.76 ml O₂ min⁻¹ kg⁻¹, which was lower than the subadult male New Zealand fur seal (13.56 \pm 2.45 ml O₂ min⁻¹ kg⁻¹; Table 3.1). The adult male Australian sea lion sSMR (10.97 \pm 1.15 ml O₂ min⁻¹ kg⁻¹) which was similar to that of adult females (11.52 \pm 3.55 ml O₂ min⁻¹ kg⁻¹), and sSMR for the subadult female was higher than mean sSMR of adult females (15.09 \pm 2.83 ml O₂ min⁻¹ kg⁻¹). For Australian fur seals the male had a lower sSMR (10.13 \pm 1.84 ml O₂ min⁻¹ kg⁻¹) than the female (12.33 \pm 1.81 ml O₂ min⁻¹ kg⁻¹). Across species the sSMR for adult female sea lions and fur seals was similar (Fig. 3.1A). The adult males of all species had similar sSMR (Fig. 3.1B). The subadult male New Zealand fur seal did not differ in sSMR from the female or male subadult Australian sea lion (Fig. 3.2), though the subadult male sea lion had a lower sSMR than the female subadult sea lion (Fig. 3.2).

3.3.1. Active (submerged swimming) metabolic rate

A total of 313 trials were conducted with 12 seals, with 272 being used for analyses. Some trials were excluded as the seals' oxygen consumption did not return to baseline. Seals swam submerged for a mean duration of 1.64 ± 0.59 minutes (range 0.7 to 3.2 minutes) and the mean mass-specific active metabolic rate (sAMR) was 25.61 ± 6.93 ml O₂ min⁻¹ kg⁻¹ (range: 12.93 to 47.60) or about 8.2 (range 4.3 to 12.7) times the predicted BMR for a terrestrial mammal of equal size.



Figure 3.1 Boxplots of standard and active metabolic rate of adult fur seals and sea lions. Median, interquartile range (box) and range (bars) of mass-specific active metabolic rate (sAMR ml $O_2 \min^{-1} kg^{-1}$ – white plots) and mass-specific standard metabolic rate (sSMR ml $O_2 \min^{-1} kg^{-1}$ – grey plots) for: A - adult female and B - adult male fur seals and sea lions.



Figure 3.2 Boxplots of standard and active metabolic rate of subadult seals. Median, interquartile range (box) and range (bars) of mass-specific standard metabolic rate (sAMR ml min⁻¹ O_2 kg⁻¹ – white plots) and mass-specific active metabolic rate (sSMR ml O_2 min⁻¹ kg⁻¹ – grey plots) for subadult fur seals and sea lions.

Table 3.1 Descriptive characteristics and summary statistics of swimming trials and metabolic rates of 12 seals. Descriptive characteristics include; species, sex, age, mass and animal ID. Mean (\pm SD) mass-specific standard metabolic rate (sSMR), mean (\pm SD) mass-specific active metabolic rate (sAMR) measured after periods of submerged swimming, mean (\pm SD) swim duration and recovery times, minimum cost of transport (COT min) and swim speed for 12 captive seals. N1 is the sample size for sSMR and N2 is the sample size for all variables calculated from a swim. Animals that were considered subadult are indicated by (*) next to age.

Sex/	Animal	Age	Mass (kg)	sSMR	N1	sAMR	Swim time	Recovery	COT min	Swim speed	N2
Species	ID	(years)		$(ml O_2 min^{-1} kg^{-1})$		$(ml O_2 min^{-1} kg^{-1})$	(min)	time (min)	(J min ⁻¹ kg ⁻¹)	(m/s)	
Arctocephalus. pusillus doriferus (Australian fur seal)											
Female	AFF1	17	71.2 (± 2.6)	12.33 (± 1.81)	6	16.65 (± 3.45)	1.57 (± 0.18)	2.26 (± 0.30)	3.21	2.82 (± 0.31)	13
Male	AFM1	15	177.4 (± 2.9)	10.13 (± 1.84)	14	15.24 (± 6.00)	1.78 (± 0.34)	3.27 (± 0.95)	2.79	2.37 (± 0.13)	33
Species m	iean		124.3	11.23		15.95	1.68	2.80	3.00	2.60	
Neophoca cinerea (Australian sea lion)											
Female	ASF1	5*	45.0 (± 0.5)	15.09 (± 2.83)	14	26.65 (± 4.16)	1.36 (± 0.12)	2.97 (± 0.64)	4.55	2.78 (± 0.51)	14
Female	ASF2	8	59.0 (± 1.6)	12.72 (± 2.62)	11	11.29 (± 6.14)	1.59 (± 0.31)	1.80 (± 0.33)	3.30	2.13 (± 0.71)	19
Female	ASF3	16	68.0 (± 1.0)	11.22 (± 3.66)	17	9.64 (± 4.80)	1.56 (± 0.38)	2.83 (± 0.67)	3.44	1.74 (± 0.34)	37
Female	ASF4	17	66.9 (± 3.6)	12.65 (± 3.38)	8	15.79 (± 4.25)	1.50 (± 0.38)	1.76 (± 0.56)	2.94	2.60 (± 0.37)	16
Female	ASF5	24	71.6 (± 1.6)	9.82 (± 3.03)	15	7.89 (± 2.75)	1.93 (± 0.22)	2.46 (± 0.77)	4.03	1.37 (± 0.20)	30
Male	ASM1	9*	110.0 (± 1.0)	10.97 (± 1.15)	13	11.06 (± 2.83)	2.04 (± 0.22)	3.39 (± 0.54)	3.83	2.34 (± 0.49)	13
Male	ASM2	12	161.4 (± 3.7)	9.84 (± 1.60)	19	15.13 (± 2.92)	1.29 (± 0.29)	2.39 (± 0.71)	2.29	1.77 (± 0.19)	32
Female n	nean		62.1	12.30		14.25	1.59	3.66	2.75	2.05	
Male mea	an		135.7	10.41		13.10	1.67	3.06	2.62	2.12	
Species n	nean		83.1	11.76		13.92	1.61	3.49	2.71	2.10	
Arctocephalus forsteri (New Zealand fur seal)											
Male	NFM1	7*	54.0 (± 3.5)	13.56 (± 2.45)	13	30.28 (± 5.44)	1.02 (± 0.25)	1.76 (± 0.53)	5.09	1.55 (± 0.26)	22
Male	NFM2	10	153.4 (± 22.0)	9.89 (± 1.92)	20	6.39 (± 3.16)	2.02 (± 0.50)	3.06 (± 0.80)	3.00	1.50 (± 0.16)	44
Male	NFM3	13	151.7 (± 17.7)	9.62 (± 1.57)	13	17.75 (± 2.51)	0.94 (± 0.10)	2.35 (± 0.49)	3.26	1.95 (± 0.12)	22
Species m	iean		152.6	11.05		18.14	1.33	2.39	3.82	1.67	



Figure 3.3. Change in mass-specific active metabolic rate (ml $O_2 \text{ min}^{-1} \text{ kg}^{-1}$) with swim duration for 12 captive seals during submerged swimming. A) four adult Australian sea lion females (filled circles); one subadult female Australian sea lion (empty circles); B) one male (filled diamonds) and one female (empty diamonds) Australian fur seals; C) one adult male (filled triangles) and one subadult male (empty triangles) Australian sea lion; D) two adult male (filled square – ASM1; crossed square – ASM2) and one subadult (empty squares) New Zealand fur seals. Fitted lines are exponential equations showing the fit for significant relationships. Equations for lines are displayed on the figure, fit parameters are available in text.

There was a significant negative relationship between swimming time and sAMR for all seals (Fig. 3A-D). The equation for the adult female sea lions was sAMR = 31.01-0.19(time) (logLik = -257.70, P < 0.001, $R^2 = 0.621$) and for the subadult female sea lion it was sAMR = 63.03-0.45(time) (logLik = -32.75, P < 0.001, $R^2 = 0.616$), the slopes of which were significantly different (z = -2.47, P = 0.013). The slopes were not different for the male and female Australian fur seals (z = 0.07, P = 0.946), therefore their data were combined. The equation for the Australian fur seals was sAMR = 35.84-0.23(time) (logLik = -76.49, P < 0.001, $R^2 = 0.773$). Australian sea lion adult and subadult male also had the same slope (z = 0.71, P = 0.478) and the equation was sAMR = 22.07-0.09(time) (logLik = -96.79, P < 0.001, $R^2 = 0.616$). The equation for the subadult New Zealand fur seal was sAMR = 45.14-0.27(time) (logLik = -60.71, P < 0.001, $R^2 = 0.455$)

and for the adults it was sAMR = 23.59-0.12(time) (logLik = -145.98, P < 0.001, $R^2 = 0.901$) and the slopes were significantly different (z = 2.36, P = 0.018).

The subadult female Australian sea lion had a higher sAMR (26.65 \pm 4.16 ml O₂ min⁻¹ kg⁻¹) than adult female Australian sea lions (11.15 \pm 4.94 ml O₂ min⁻¹ kg⁻¹). The subadult male and adult Australian sea lion were similar, (Fig. 3.2), while the mean sAMR for the subadult male New Zealand fur seal (30.28 \pm 5.44 ml O₂ min⁻¹ kg⁻¹) was higher than that of adults (12.07 \pm 1.66 ml O₂ min⁻¹ kg⁻¹). The adult male and female sea lions had very similar sAMR (Fig. 3.1), as were the male and female Australian fur seal (Fig. 3.2). The adult females and the adult males of all species had similar sAMR (Fig. 3.2).

3.3.2. Relationship with speed

The variation between sAMR and speed for four adult female sea lions was best explained by the equation sAMR = $3.72\exp(0.54 \times \text{speed})$ (logLik = -246.28, $R^2=0.59$, n = 91). For the youngest female sea lion (ASF1) the equation was sAMR = $15.38\exp(0.20 \times \text{speed})$ (logLik = -32.29, $R^2=0.63$, n = 13; Fig. 3.4A). The relationship between COT and speed for four adult female sea lions (Fig. 3.5A) was best described by COT = $2.85\exp(\text{speed}^{-1.16})$ (logLik = -115.79, $R^2 = 0.61$, n = 91) and by COT = $11.16\exp(\text{speed}^{-0.25})$ for ASF1 (logLik = -0.93, $R^2 = 0.85$, n = 13; Fig. 3.5A).

There was no relationship between sAMR and speed for the subadult or adult male sea lions (Fig. 3.4C). The relationship between COT and speed for male sea lions were different. The relationship for the subadult male Australian sea lion (ASM1) was: $COT = 14.75exp(-0.47 \times speed)$ (logLik = -10.70, $R^2 = 0.66$, n = 14) and for the adult male Australian sea lion (ASM2): $COT = 13.59exp(-0.71 \times speed)$ (logLik = -20.97, $R^2 = 0.53$, n = 29; Fig. 3.5C).

The adult male New Zealand fur seals data were unable to be pooled for investigation as they swam for very different durations, despite being similar age and mass in both trials. The variation between sAMR and speed for two adult male New Zealand fur seals (NFM1 and NFM2) could not be reliably predicted. The relationship for the subadult male New Zealand fur seal (NFM3) was: sAMR = $17.42\exp(0.33\times\text{speed})$ (logLik = -44.49, $R^2 = 0.47$, n = 15) (Fig. 3.4D). The relationship between COT and speed for the long swimming adult male New Zealand fur seal (NFM2) was best described by COT = $37.31\exp(\text{speed}^{1.06})$ (logLik = -61.68, $R^2 = 0.36$, n = 42) and by COT = $3.05\exp(\text{speed}^{-2.93})$ for the short swimming adult male New Zealand fur seal (NFM2) was best described by COT = $3.52\exp(\text{speed}^{-1.06})$ (logLik = -2.13, $R^2 = 0.82$, n = 13; Fig. 3.5D). The relationship between COT and speed for the subadult male New Zealand fur seal (NFM3) was best described by COT = $3.16\exp(\text{speed}^{-0.53})$ (logLik = -22.49; $R^2 = 0.19$; n = 15; Fig. 3.5D).

The relationship between speed and sAMR for the female adult Australian fur seal (AFF1) fitted the equation: sAMR = $5.75\exp(0.39 \times \text{speed})$ (logLik = -32.06, R²=0.37; n = 13; Fig. 3.4B) and by: sAMR = $0.16\exp(1.93 \times \text{speed})$ (logLik = -78.33, R²=0.62; n = 28; Fig. 3.4B) for the adult male Australian fur seal (AFM1). The relationship between and speed and COT could be expressed by the equation: COT = $7.13\exp(-0.27 \times \text{speed})$ (logLik = -20.18; R² = 0.46; n = 28; Fig. 3.5B) for the adult male Australian fur seal

(AFM1) and by: $COT = 12.65exp(-0.43 \times speed)$ (logLik = -4.20; $R^2 = 0.48$; n = 13; Fig. 3.5B) for the adult male Australian fur seal (AFM1).



Figure 3.4 Change in mass-specific active metabolic rate (ml O_2 min⁻¹ kg⁻¹) with swimming speed for 12 captive seals during submerged swimming. A) four adult Australian sea lion females (filled circles); one subadult female Australian sea lion (empty circles); B) one male (filled diamonds) and one female (empty diamonds) Australian fur seals; C) one adult male (filled triangles) and one subadult male (empty triangles) Australian sea lion; D) two adult male (filled square – ASM1; crossed square – ASM2) and one subadult (empty squares) New Zealand fur seals. Fitted lines are exponential equations showing the fit for significant relationships. Equations for lines are displayed on the figure, fit parameters are available in text.

3.4. Discussion

In this study, we investigated the effects of species, development stage, sex and activity on metabolic rate, and found that differences among species were not pronounced, indicating that the three species of otariid that occupy southern Australia have similar metabolic rates. Female Australian sea lions and Australian fur seals had a higher metabolic rate per unit mass than males of the same species, as did subadult animals compared to adults. Activity significantly influenced metabolic rate for all size and taxa sampled, such that metabolic rate increased with swim speed and decreased with swim duration. Sea lions and fur seals in this study had an optimal speed at which to swim of between 1.5 and 2m/s, and this closely resembles swimming speeds found for wild otariids (Watanabe *et al.* 2011).



Figure 3.5 Change in cost of transport (COT J kg⁻¹ m⁻¹) with swimming speed for 12 captive seals during submerged swimming. Fitted lines are exponential equations showing the fit for: A) four adult Australian sea lion females (filled circles); one subadult female Australian sea lion (empty circles); B) one male (filled diamonds) and one female (empty diamonds) Australian fur seals; C) one adult male (filled triangles) and one subadult male (empty triangles) Australian sea lion; D) two adult male (filled square – ASM1; crossed square – ASM2) and one subadult (empty squares) New Zealand fur seals. Fitted lines are exponential equations showing the fit for significant relationships. Equations for lines are displayed on the figure, fit parameters are available in text.

3.4.1. Cost of swimming

We found that metabolic rate decreased with swim duration for all seals, despite not diving to depth (Fig. 3.3). Oxygen consumption rate and total body oxygen stores (TBOS) predict the depths and durations that seals can reach (Davis and Williams 2012). Theoretically, this can be estimated as the calculated aerobic

dive limit (cADL) which is estimated from TBOS and the rate with which they are depleted by metabolic processes, most commonly estimated with diving metabolic rate (DMR) (Shero *et al.* 2012, Gerlinsky *et al.* 2013). cADL is therefore the amount of time that an air-breathing animal can theoretically dive without the use of anaerobic metabolism (Costa *et al.* 2001). Dive durations can be maximized through the dive response in which heart rate is reduced (bradycardia), resulting in a lower rate at which total oxygen stores are consumed (Davis and Williams 2012). Pinnipeds display bradycardia while diving, the extent of which differs among species (Ponganis *et al.* 1991, Hindle *et al.* 2010, McDonald and Ponganis 2014). As bradycardia should preserve lung oxygen stores (McDonald and Ponganis 2014), the aerobic dive limit should increase, increasing the duration of dives. Despite swimming in shallow pools, all the animals in our study demonstrated a decrease in metabolic rate with extended submerged periods, indicating that the seals may have exhibited a dive response.

Water density and viscosity are major factors that contribute to the morphology of marine mammals (Williams 1999) and whilst diving, marine mammals are not only resisting tonnes of pressure, but they must also contend with the physical forces of buoyancy and drag (Butler and Jones 1997). Fur seals and sea lions have evolved streamlined bodies that are neutrally buoyant in water, which significantly reduce drag and increase swimming power (Feldkamp 1987). The body sizes of the species measured in this study were significantly different, and the animals were swimming in shallow pools; therefore minimum COT is a useful measure of energy use across species as it is a measure of the efficiency of the transport process (Feldkamp 1987).

The predictive equation for cost of transport for marine mammals ranging from 21 to 15,000kg, COT (J kg⁻¹ m⁻¹) = 7.79 Mb^{-0.29}, (Williams 1999) underestimated COT for the all fur seals and sea lions (0.5 to 0.8 times predicted). The allometric equation adapted for pinnipeds and cetaceans, COT = 9.54 Mb^{-0.29} (Rosen and Trites 2002), was a better predictor of COT for adult female fur seals (0.9 times predicted) and adult male and female sea lions (1.0 times predicted). It underestimated all other groups by a factor of 0.6 to 0.8. The differences in the predictive abilities of the Williams (1999) and Rosen and Trites (2002) equations likely arise from their respective samples. Williams (1999) developed an equation based on a diverse group of marine mammals including cetaceans, pinnipeds and sirenians. Rosen and Trites (2002) focussed on pinnipeds which included male pinnipeds, and as a result of the narrower sample selection it more closely aligned to the values obtained in this study. Males tend to have lower metabolic rates than females (this study; Hurley and Costa 2001), which would account for the over-prediction in the Williams (1999) equation.

COT for pinnipeds is a substantial component of energy expenditure during their time at sea, as foraging grounds can often be far from breeding colonies. For Australian sea lions and New Zealand fur seals COT decreased exponentially as their speed increased. This relationship plateaued after 2 m/s in adult seals, but continued to decline in younger seals. Despite having different foraging strategies, the COT for adult seals in the study varied little between species. Instead differences in COT could be accounted for by

development stage (subadult or adult) or body size. At a given speed, younger seals' COT was elevated compared to adult seals of the same species and sex. Minimum COT was similar for subadult Australian sea lions and New Zealand fur seals (3.5 and 4.1 J kg⁻¹ m⁻¹ respectively) and subadult Steller sea lions (3.5-5.3 J kg⁻¹ m⁻¹ (Rosen and Trites 2002)). This was higher than minimum COT of the adult Australian fur seal (2.3 J kg⁻¹ m⁻¹), Australian sea lion (2.5 J kg⁻¹ m⁻¹) and New Zealand fur seal (2.6 J kg⁻¹ m⁻¹) which was similar to subadult CSL (2.3-2.8 J kg⁻¹ m⁻¹ (Williams 1999); 2.3 J kg⁻¹ m⁻¹ (Williams *et al.* 1991)) and adult Southern sea lion (2.6 J kg⁻¹ m⁻¹ (Dassis *et al.* 2012)) swimming at comparable speeds.

Our results suggest that, as predicted, younger seals have a higher cost of travelling (on a mass-specific basis) than older seals. Australian sea lion juveniles swim at slower speeds and travel shorter distances than adults, targeting benthic prey in shallow nearshore waters (Fowler *et al.* 2007a). This contrasts with New Zealand fur seal juveniles, which travel much greater distances, but at slower speeds than adults. New Zealand fur seal juveniles may use this strategy to target higher energy pelagic prey higher in the water column, avoid intra-specific competition (Page *et al.* 2006, Jeglinski *et al.* 2013) or because their diving depths are limited by underdeveloped total body oxygen stores (Verrier *et al.* 2011) and high metabolic rates (this study). Given the inverse relationship between COT and speed where slower speeds correspond to a higher COT, young fur seals and sea lions are particularly susceptible to resource limitations (Horning and Trillmich 1997b, Fowler *et al.* 2006).

The lack of difference between adults of the three species in COT suggests that the foraging strategies of the three species may have evolved more from reducing competition rather that physiological constraints. Typically, the benthic dives of otariids are of a greater duration and depth than pelagic dives, presumably requiring more energy and a larger body mass (Costa *et al.* 2004). Australian fur seals are the largest of the fur seals and they have adopted a benthic diving strategy which is atypical for a fur seal (Arnould and Costa 2006). The large body mass, and thus larger oxygen stores, may have evolved in order to use this strategy and avoid competition for prey with the New Zealand fur seals through diving deeper or longer (Page *et al.* 2005a). The physiological capacity to make long and deep dives may also have arisen in response to the characteristics of the habitat in which the animals reside. For example, Hückstädt *et al.* (2016) demonstrated that the foraging habitat of South American sea lions was more important than body size in predicting their diving capabilities.

Our results suggest that the optimal swimming speed for fur seals and sea lions lies between 1.5 and 2 m/s. Though we did not see very slow speeds, the seals in this study did reach higher speeds than in Williams (1999) and Rosen and Trites (2002), emphasising that at higher speeds the COT varies little. It is predicted that at very high speeds COT would increase again, creating a U shape distribution (Rosen and Trites 2002). This response plays an important role in thermoregulation in swimming mammals, whereby the increase in metabolic heat generated by activity can offset the costs of increasing the metabolic rate in cold water (Hind and Gurney 1997, Liwanag *et al.* 2009). This distribution is expected in part from thermoregulatory maintenance that marine mammals must undergo while swimming (Borgwardt and Culik 1999).

We found that fur seals and sea lions experienced a curvilinear increase in metabolic rate with increased speed (Fig. 3.4), similar to juvenile California sea lions (Liwanag *et al.* 2009). At low speeds (<1 m/s) marine mammals need to use their metabolism to maintain body heat, but while swimming at speed the body can generate excess heat from activity, which it uses to remain thermo-neutral (Hind and Gurney 1997, Liwanag *et al.* 2009). At high speeds COT increases exponentially as the major source of effort becomes overcoming hydrodynamic drag (Schmidt-Nielsen 1997). The predicted optimal speed of between 1.5 and 2 m/s is comparable to the observed travelling (0.6 to 1.9 m/s) and diving (0.9 to 1.9 m/s) speeds of wild otariids (Ponganis *et al.* 1990, Ponganis *et al.* 1992, Watanabe *et al.* 2011). This suggests that while foraging otariids travel at close to their optimal speed, and therefore comply with predictions about foraging efficiency.

There was a considerable spread of values in the metabolic rates estimated. These potential sources of error come from a number of sources including: measurement error, temperature (discussed in Chapter 2), body size and the swimming trajectories of the seals. We performed extensive data exploration to remove any obscure measurements that arose due to measurement error or a behavioural anomaly. However, there may still be cases that have escaped detection. We tested for the effect of temperature in each relationship and found no significant impact. However, this effect may have been diluted by the activity, which could potentially be a large source of error. While we attempted to replicate the swimming conditions at each institution, there were inevitable differences in the distance swum before a turn (impacting the number of turns an individual completed in a trial) as well as the depth of the swim. Depth may impact the swimming effort as below a certain point the effects of buoyancy are subdued (Butler and Jones 1997), where at shallower depth there is a greater drag effect. Turning has also been shown to be a large part of movement energetics as it increases resistance and the distance travelled (Fish et al. 2003), thus may cause of some of the variation in metabolic rates . Effects of development and sex on standard and active metabolic rate

Despite pronounced differences in foraging strategy and body size we found very little evidence of differences in metabolic rates among the three species of otariid. Groups of seals with different foraging strategies and prey types are expected to have different metabolic rates or oxygen carrying capacity (Mori 2002, Costa *et al.* 2004, Arnould and Costa 2006). Australian fur seals and sea lions adopt a benthic strategy, targeting benthic or demersal prey, that require long dive durations and bottom times to find and capture (Arnould and Costa 2006, Fowler *et al.* 2006, Lowther *et al.* 2011, Lowther *et al.* 2013). New Zealand fur seals typically forage pelagically, targeting vertically migrating prey that occur in the upper mixing layer of water (<50m depth) (Harcourt *et al.* 2002, Page *et al.* 2005a). This strategy is expected to be less energetically costly as dives are typically shallower and shorter than benthic dives.

The Australian fur seal and sea lion are much larger than the New Zealand fur seal, and a larger body size implies a larger oxygen carrying capacity. Female Australian fur seals and sea lions have very similar oxygen stores: 46 and 47 ml O_2 kg⁻¹ respectively (Costa *et al.* 2001, Spence-Bailey *et al.* 2007). A single measurement of New Zealand fur seals has been made, where their O_2 capacity was estimated at 22.6 ml/100ml (Wells 1978). Assuming a total blood volume of 17.8L (northern fur seal (*Callorbinus ursinus*) (Lenfant *et al.* 1970))

and a body mass of ~150kg (Table 3.1) we estimate total O_2 stores of male New Zealand fur seals at 26.9 ml O_2 kg⁻¹. Using a similar metabolic rate would imply that New Zealand fur seals would use up their stores much quicker than either the Australian fur seal or Australian sea lion. New Zealand fur seals typically dive for between 1.2 and 2.7 minutes (Harcourt *et al.* 1995, Page *et al.* 2005b), while the Australian fur seal has an average dive duration of 3.2 minutes (Hoskins *et al.* 2015) and the Australian sea lion dives for an average of 3.3 minutes (Costa and Gales 2003) (Fowler *et al.* 2006). This implies that despite having similar metabolic rates, the oxygen carrying capacity differs between the benthic and the pelagic divers, resulting in different dive durations and very likely influencing dive strategies (Costa *et al.* 2004, Arnould and Costa 2006).

The subadults of Australian sea lions and male New Zealand fur seals demonstrated elevated COT over a range of swimming speeds (Figs. 3.5A, C, D) and elevated sSMR (measured at rest – except ASM1). Elevated metabolic rates in immature animals are likely to be associated with the additional cost of growth as they synthesise and store new tissue in the body (Donohue *et al.* 2000), though the pattern of decline to adult levels differs between and within phocid and otariid species. For example, juvenile grey seals (*Halichoerus grypus*) demonstrate a resting metabolic rate (RMR) that is 35% higher on average than predicted for adult grey seals of a similar mass, with their overall RMR declining as they reach sexual maturity around 3 to 5 years of age (Boily and Lavigne 1997). In comparison, the RMR of juvenile and adult California sea lions did not differ over a range of temperatures (Liwanag *et al.* 2009). Similarly, there were no differences in the metabolic rates of subadult male and adult female Southern sea lions (Dassis *et al.* 2012). Our results align with grey seal metabolic development, as the sSMR and sAMR of two of our subadults were still higher than the adults at five and seven years of age.

Juvenile and subadult otariids tend to travel shorter distances, perform shallower dives and dive for shorter durations than adults of the same species (Horning and Trillmich 1997a, Fowler *et al.* 2006, Lowther *et al.* 2013). Juvenile New Zealand fur seals and Australian sea lions forage for shorter durations and to shallower depths than their adult counterparts (Fowler *et al.* 2006, Page *et al.* 2006). At 23-months Australian sea lions only had 70% of adult total oxygen stores and 78% at three years (Fowler *et al.* 2007b). This, coupled with the high metabolic rates when subadult found in this study, would explain why they only reached 76% of adult depths and 77% of their durations (Fowler *et al.* 2006). Similarly, juvenile New Zealand fur seals exploit shallower waters than their adult counterparts (Page *et al.* 2006). Subadult New Zealand fur seals would have an additional constraint on foraging at depth in comparison to adults, as having a higher metabolic rate means that their energy resources are used much quicker. Young otariid seals will opt to decrease their physical activity to conserve energy rather than spending more time searching for food (Verrier *et al.* 2011). This may mean that until subadult age otariid seals are susceptible to environmental changes that affect prey abundance.

Any sex differences in the metabolic rates of pinnipeds are predicted to be as a result of the vastly different roles they occupy for breeding. It is expected that as adult females are usually pregnant and/or lactating, requiring a constant supply of energy throughout the year, they will have a higher metabolic rate than males,

who only bulk up for the breeding season, maintaining a lower body mass for the remainder of the year (Costa 1991a, Williams *et al.* 2007). In this study we were able to remove the effect of breeding to test if this was a significant effect on the metabolic rates of male and female otariids. When comparing adult males and females of the same species, females had a higher sAMR and sSMR than males. Sex differences in metabolic rate and oxygen stores are inconsistent across pinnipeds. In Californian sea lions, females have a higher mass-specific metabolic rate and mass-specific total oxygen stores than males (Hurley and Costa 2001, Weise and Costa 2007), and Antarctic fur seal and grey seal females have a higher mass-specific metabolic rates than the males (Boyd and Duck 1991, Rosen and Renouf 1995, Boyd and Croxall 1996). In contrast, in Southern sea lion sex did not have a significant effect on mass-specific metabolic rate, though the comparison was made between subadult males and adult females (Dassis *et al.* 2012). It is unclear in these studies whether the seals that were sampled were reproductive, but we have shown that mass-specific metabolic rates are higher in non-reproductive female seals than males. Fur seals and sea lions are sexually dimorphic, where males may be 4 times the size of the females (Lindenfors *et al.* 2002) and it is expected that smaller bodied animals will have higher metabolic rates (Kleiber 1975). Therefore, any differences that are evident are likely due to allometry, rather than any additional costs of reproduction.

3.4.2. Conclusion

During activity, Australian fur seals, New Zealand fur seals and Australian sea lions all appear to be operating at their physiological optimum. Minimum COT occurs at speeds typically measured from foraging wild otariids. There were no species differences in COT when comparing adults of the same sex. There were some intra-specific differences however. As expected, younger seals have additional energetic cost associated with activity. Though this cost may be carried later in life than for other seals as we measured subadult animals. The higher mass-specific metabolic rate observed in females is likely driven by extreme sexual dimorphism. Therefore, young and female seals may experience physiological constraints should they need to increase their foraging effort in response to changing prey distributions.

Chapter 4

Seeing it all: evaluating supervised machine learning methods for the classification of diverse otariid behaviours

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Abstract

Constructing activity budgets for marine animals when they are at sea and cannot be directly observed is challenging, but recent advances in bio-logging technology offer solutions to this problem. Accelerometers can potentially identify a wide range of behaviours for animals based on unique patterns of acceleration. However, when analysing data derived from accelerometers, there are many statistical techniques available which when applied to different data sets produce different classification accuracies. We investigated a selection of supervised machine learning methods for interpreting behavioural data from captive otariids (fur seals and sea lions). We conducted controlled experiments with 12 seals, where their behaviours were filmed while wearing 3-axis accelerometers. From video we identified 26 behaviours that could be grouped into one of four categories (foraging, resting, travelling and grooming) representing key behaviour states for wild seals. Data from ten seals were used to train four predictive classification models: stochastic gradient boosting (GBM), random forests, support vector machine using four different kernels and a baseline model: penalised logistic regression. We then took the best parameters from each model and cross-validated the results on the two seals unseen. The influence of feature statistics (describing some characteristics of the seal) was investigated. Cross-validation accuracies were lower than training accuracy, but the support vector machine (SVM) with a polynomial kernel classified seal behaviour with high accuracy (>70%). Adding feature statistics improved accuracies across all models tested. Resting, grooming and feeding behaviours were all predicted with reasonable accuracy (52-81%) by the SVM, while travelling was poorly categorised (3141%). These results show that model selection was important when classifying behaviour and including animal characteristics improved the overall accuracy.

Key words

Accelerometry, otariid, activity budgets, machine learning

4.1. Introduction

Advances in bio-logging technologies have provided a means by which we can accurately quantify the activity budgets of marine predators (Cooke *et al.* 2004, Hussey *et al.* 2015). Previously, investigators have used multiple devices and/or direct observation to investigate a single parameter (e.g. feeding; Viviant *et al.* 2010, Skinner *et al.* 2014). Observation allows researchers to record detailed behaviour without directly interacting with the animal, though this method is often inefficient due to the inability of researchers to record behaviour at all times and is biased to observations at or near the surface (Martin and Bateson 1993). In addition, marine predators are difficult if not impossible to observe in the wild as they spend most of their time underwater and can forage over great distances (Cooke *et al.* 2004). Well documented observer effects add to the limitations of direct observation, and this has lead researchers to develop devices that allow us to record animal behaviour remotely (Brown *et al.* 2013).

Time-depth recorders and stomach temperature loggers have been used in combination to predict when an animal has captured and ingested prey (Andrews 1998). However, gaining complete information from a multi-instrument approach can be invasive, expensive, analytically complicated and is not always successful (Austin et al. 2006). A more refined approach is to use devices that can measure physical activity over periods long enough to be representative of typical daily activities, with minimal discomfort to the animals, and applicable to large populations (Westerterp 2009). Tri-axial accelerometers are one option, as these can measure animals in their natural environments over long periods and in places where observation is difficult or impossible (Cooke et al. 2004, Carroll et al. 2014). These devices are increasing in popularity and offer opportunity to study marine predators with a level of detail that other devices do not (Halsey et al. 2011a). They allow us to measure and classify the activity of animals using data from a single device (Watanabe et al. 2005), and can be incorporated into more complex devices along with sensors that record physical and environmental parameters such as depth and temperature (Wilson et al. 2008). Unique combinations of the three accelerometry axes; heave, surge and sway, can be used to identify different activities (Halsey et al. 2011a). Feeding events can be identified from mandible and head mounted accelerometers (Sakamoto et al. 2009, Viviant et al. 2010, Ydesen et al. 2014), but a wider range of behaviours, and a proxy for the energy expenditure of those behaviours, may be predicted from mounting the device close to the mid-point of the animals torso (Halsey et al. 2009b).

Currently many methods and techniques exist for the classification of accelerometry data. Supervised and unsupervised algorithms provide options for classification and interpretation (Sakamoto *et al.* 2009,

Nathan *et al.* 2012). Supervised learning can adjust its classifications by using error messages programmed by the user, whereas unsupervised learning looks for patterns in the data. Supervised learning requires the input of a 'teacher' to manually classify the behaviour to 'teach' the program how to identify each behaviour (Sathya and Abraham 2013). This method is highly accurate and precise, but is also very time consuming. In contrast, unsupervised learning classifies behaviour using heuristics, and has the advantage of speed, which it trades for accuracy or precision (Sathya and Abraham 2013). It may also pick up patterns in the data that manual classification methods do not. When classifying data for supervised learning there is a degree of subjectivity involved on behalf of the teacher, whereas unsupervised learning algorithms classify data with an unbiased view (Brown *et al.* 2013).

Published ethograms have used a wide variety of these methods with varying degrees of success, including quadratic discriminant analysis (QDA) for the classification of activity in cattle and humans (Pober *et al.* 2006), decision trees with turtles (Nishizawa *et al.* 2013) random forests with badgers (McClune *et al.* 2014, Graf *et al.* 2015), and neural networks with humans (Yang *et al.* 2008). Each method has advantages and disadvantages, and it is likely that different methods will work better for different species, device placement and settings. With the significant advancement of computer speed and the relative ease with which these methods can be implemented an important step is to determine the most appropriate method of analysis for the particular set of circumstances under study.

To explore this, we used data from captive otariid pinnipeds to assess the reliability of a number of different machine learning algorithms in identifying particular behaviours. Activity budgets of otariids include activity on land and in water, and water behaviours can be more complex to define as they involve dynamic movement in a 3D environment. To date, quantifying pinniped behaviour using accelerometers has focussed on identifying foraging and travelling behavioural states (Naito *et al.* 2010). Less attention has been paid to other potentially important behaviour states, such as grooming, reproductive and resting behaviours, despite these being major components of their behavioural repertoire and possible indicators of important underlying indicators such as condition (Iwata *et al.* 2013, Battaile *et al.* 2015). As yet, no studies have sought to quantify the terrestrial behaviours displayed by pinnipeds using accelerometers. The aims of this paper were (1) to build a detailed ethogram of the key behaviours performed by captive otariid pinnipeds, applicable to wild populations, and (2) to use a range of machine learning algorithms to classify these behaviours, providing us with the opportunity to test and compare the accuracy of these different methods.

4.2. Materials and Methods

4.2.1. Animals

We conducted experiments with two Australian fur seals (*Arctocephalus pusillus doriferus*), three New Zealand fur seals (*Arctocephalus forsteri*), one subantarctic fur seal (*Arctocephalus tropicalis*), and six Australian sea lions (*Neophoca cinerea*) (Table 4.1), from three Australian marine facilities: Dolphin Marine Magic,

Coffs Harbour (RF1: -30°17'N, 153°8'E); Underwater World, Sunshine Coast (RF2: -25°40'N, 153°7'E); and Taronga Zoo, Sydney (RF3: -33°50'N, 151°14'E). Experiments were conducted from August to November 2014 at all three institutions, and again in August 2015 at RF2. The seals were on permanent display at their respective marine facilities and were fed and cared for under the guidelines of the individual facility. All Australian sea lions in the study were born as a part of an ongoing captive breeding program in Australian aquaria, while all fur seals came into captivity as juveniles, in poor health or injured, and were considered unsuitable for release. All fur seals were in very good health during the study. This study was conducted under permits from Macquarie University ethics committee (ARA-2012_064) and Taronga ethics committee (4c/10/13).

Table 4.1. Identification number, location, species, age, mass and sex of seals with number of sessions and attachment method of accelerometer. AFS - Australian fur seal; NZFS - New Zealand fur seal; SFS – subantarctic fur seal and ASL - Australian sea lion.

Seal ID	Marine facility	Species	Age	Mass range (kg)	Sex	Number of sessions	Attachment method
ASF1	RF1	ASL	5	44-47	Female	13	Harness
ASF3	RF2	ASL	17	58-74	Female	4	Harness
ASF4	RF1	ASL	17	66-70	Female	12	Harness
ASF6	RF1	ASL	7	50	Female	2	Harness
ASM1	RF1	ASL	9	108-110	Male	8	Harness
AFF1	RF2	AFS	17	69-79	Female	7	Таре
AFM1	RF2	AFS	16	175-242	Male	7	Таре
ASM2	RF3	ASL	13	160-162	Male	9	Tape
NFM1	RF3	NZFS	8	47-54	Male	5	Таре
NFM2	RF2	NZFS	11	108-152	Male	5	Tape
NFM3	RF3	NZFS	13	111-154	Male	8	Таре
SFM1	RF2	SFS	4	28-30	Male	3	Таре

4.2.2. Experimental protocol

Seals were fitted with a tri-axial accelerometer (CEFAS G6a+: 40mm x 28 mm x 16.3 mm,18 g in air and 4.3 g in seawater, CEFAS technology Ltd, Lowestoft, UK) positioned between the shoulder blades. Accelerometers recorded three axes of acceleration: surge (x-axis), sway (y-axis) and heave (z-axis). They were orientated such that the x-axis was anterior–posterior, the y axis was lateral and the z axis was dorsal–ventral. Accelerometers recorded at +-8g, at a rate of 25 samples per second (25Hz), and logged wet/dry events.

For fur seals, accelerometers were secured between the shoulder blades on the top layer of fur using Tesa tape (Tesa, Eastern Creek, NSW, Australia; Fig. 4.1). The process took around two minutes to

attach and 30-60 seconds to remove. This method could not be used for the sea lions as the fur was too short for the tape to hold the devices. Instead, we used a custom designed harness (©Guy Bedford) with three clips, one around the neck and two at the back (Fig. 4.2), and accelerometers were fitted into a pocket sewn to the back.



Figure 4.1 Process of accelerometer attachment with tape. a) Dry the fur; b) Lift the hair to stick tape to undercoat; c-e) Tape on the accelerometer; f) Seal with accelerometer



Figure 4.2 Harness. a) Back; b) Side; c) Front

Each session was recorded using two or three cameras filming at 50 frames per second (FPS); one or two cameras (GoPro Hero 3 – Black edition, USA) were placed in a pool below the water line to capture all underwater behaviour and above water behaviour was captured by a hand held camera (HDRSR11E: Sony, Japan). We used different pools depending on the facility. At RF1 we used three pools, the first pool was 11m diameter and 3m deep, the second pool was 12m wide, 24m long and an average depth of 2m, the third pool was 7m diameter and 2m deep. At RF2 we used one large pool which was 11m wide, 14m long with an average depth of 8m. At RF3 we used three pools, the first was 6m wide, 15m long and an average of 3m deep, the second pool was 9m wide, 12m long and an average of 3m deep, the third pool was 26m long, 9m wide and 5m deep. We defined a session as a continuous period that seals were wearing the accelerometer and being filmed, and we attempted only one session per day per seal.

Sessions had a maximum duration of 90 minutes after which the accelerometer removed and the seal was rewarded. Seals participated in 3-11 sessions.

We observed seals during training sessions where behaviours were requested using operant-conditioning, and without being asked to perform specific behaviours. Seals were not restrained or required to give a behaviour. We observed two types of sessions; feeding and behaviour sessions. The feeding sessions aimed to provide seals with large food items that required some form of processing prior to eating (see Hocking *et al.* 2015). Seals were given a range of seafood including bream (*Abramis brama*), mullet (*Mugil cephalus*), Sydney octopus (*Octopus tetricus*), Australian salmon (*Arripis truttaceus*), mackerel tuna (*Euthynnus affinis*), New Zealand brill (*Colistium guntheri*) and yellowtail amberjack (*Seriola lalandi*). Seals entered the water and were given the particular food item in the water with an unrestricted amount of time to eat. When a seal did not eat the food either another seal was introduced to the pool to encourage competition, or the original seal was returned to its pen and a different seal was fitted with an accelerometer and presented with the food.

Behaviour sessions also incorporated some feeding events with small fish that did not require processing. Fish were thrown in the pool so that seals had to capture them mid-water as they sank. During each behaviour session seals were instructed to perform a series of natural behaviours from their known behavioural repertoire (Table A-1). These behaviours were expected to emulate the behaviour of wild seals, such as porpoising, swimming and grooming. Behaviours were repeated during a session until the food was exhausted or the seal did not respond to instruction.

4.2.3. Statistical analyses

4.2.3.1. Data preparation

The acceleration data were downloaded using the G5 Host software (Version 6.4 CEFAS Technology Ltd). The video from each camera was imported into Adobe Premiere Pro CC (Adobe Systems Inc., California) where it was synchronized so that the video files could be easily viewed together. They were then exported at 25 FPS as a single movie file. Data were coded manually using Excel (Microsoft Corp., Washington, USA) and QuickTime (Apple Computer Inc., California, USA). To synchronise the accelerometer and the video, we "marked" the accelerometer on the video by hitting it against a hard surface while filming. This caused a large spike in the accelerometry data that we could match exactly to the video. We matched each accelerometry data sample with the corresponding video frame and the specific behaviour recorded in Excel (see Table A-1 for a detailed list of behaviours and their descriptions). Videos were scored without interruption.

The duration of a behaviour ranged from 0.25 (e.g. shake) to 3.5 minutes (e.g. continuous swimming). We coded 26 unique behaviours, but because there were not enough samples of each of the individual behaviours, we grouped behaviours into five categories. These behaviour categories were chosen based on a combination of ecological and behavioural knowledge of the target species, rather than on statistically identifiable behaviours (as in unsupervised learning). The five categories were grooming, travelling, foraging, resting and other. The 'other' category consisted of direct feeding by the trainer

(when the food was delivered by hand or thrown and caught), behaviours that could not be clearly placed into one of the other categories, and time where the seal was out of sight. As these cannot be considered natural behaviours, accelerometry data collected at these times was not included in the analysis. Where behaviours overlapped, or were displayed simultaneously (e.g. foraging and travelling), grooming and foraging took precedent over travelling and resting. Half of the videos were coded by two coders (JK and ML) and compared for validation. The coders recorded the same behaviour in over 95% of cases, therefore the first coder (JK) completed the remaining coding.

Data were summarised into epochs (sliding sample windows) of 13 samples which represented approximately 0.5 sec data. This would ensure that the shortest recorded behaviour would be captured. Data were further split into training and validation sets, where data from ten seals were used for training and data from two seals were kept as an out-of-sample set for cross-validation of the models. This was to assess the models true generalisability (Gerencser *et al.* 2013).

4.2.3.2. Summary statistics

Choosing the number of summary statistics that are put into a model can be highly subjective. Complex behaviours, and large numbers of example behaviours means that a large number of summary statistics are likely required. A greater number of summary statistics improves the algorithms chances of detecting subtle differences between the behaviours (Shepard et al. 2008b, Brown et al. 2013). We coded 52 summary statistics and added five feature statistics describing some characteristic of the individual or the event to the second stage of model testing. These were included to assess their overall impact on prediction performance of the models. The features we included were device attachment method (harness or tape), age, mass, sex and species of the individual. We included where the behaviour occurred (surface, underwater or land) in all models. We calculated summary statistics including mean, median, standard deviation, skewness, kurtosis, minimum, maximum, absolute value, inverse covariance and autocorrelation trend (the coefficient derived from a linear regression) for each of the three axes. We also calculated q as the square-root of the sum-of-squares of the three axis (Nathan et al. 2012), and included pair-wise correlations of the three axis (x-y, y-z, x-z) (Ravi et al. 2005). The inclination as azimuth were calculated as per Nathan et al. (2012). We calculated three measures of dynamic body acceleration (DBA) by first using a running mean of each axis over 3 seconds to create a value for static acceleration. We then subtracted the static acceleration at each point from the raw acceleration value to create a value for partial dynamic body acceleration (PDBA). The values of PDBA on each axis were summed to calculate overall dynamic body acceleration (ODBA) (Wilson et al. 2006, Shepard et al. 2008a). We calculated VeDBA (Eq. 2) as the square root of the squared PDBA of the three axis (Qasem et al. 2012) and calculated the area under the curve for both ODBA and VeDBA using the package "MESS" in R (Ekstrom 2014, R Core Development Team 2015).

$$ODBA = |X_{dyn}| + |Y_{dyn}| + |Z_{dyn}|$$
(4.1)

$$VeDBA = \sqrt{X_{dyn}^2 + Y_{dyn}^2 + Z_{dyn}^2}$$

4.2.4. The models

4.2.4.1. Penalised logistic regression

In logistic regression the probability of each outcome was estimated via a logistic function which transformed a binary [0, 1] outcome to a continuous outcome from negative infinity to positive infinity. A linear relationship was then found between the transformed outcome and the input variables (this process was performed in one step, but is easier to visualise as a two stage process). A penalty was added to the error function of this process to avoid over fitting of the problem. Common forms of this penalty are either the L1 or L2 norm. In effect this penalty shrinks the coefficients of the logistic regression towards zero, to simplify the model. We implemented logistic regression to set a base line accuracy against which the other, more complicated models were compared. The penalised logistic regression was implemented using the R package "glmnet" (Friedman *et al.* 2010).

4.2.4.2. Support vector machines

Support vector machines (SVM) are a form of discriminant classifier, where this discrimination was performed by hyperplanes that divide the input data into classes according to their labels (Cortes and Vapnik 1995). In essence two hyperplanes were employed and the distance between them chosen to maximise the distance between the two classes. Hence a SVM is often referred to as a maximal margin classifier. The simplest form of SVM used a linear kernel to find a way to linearly separate the classes. Often the data do not separate linearly in which case nonlinear kernels were used to map the features to different vector spaces where it may be possible to better separate the data. We tested linear, polynomial, radial and sigmoid kernels. The SVM was implemented using the R package "e1071" (Meyer *et al.* 2015).

4.2.4.3. Random forests

Random forests are a form of ensemble learning (Breiman 2001). An ensemble is a combination of different classifiers (referred to as base learners) each trained to perform the same classification, generally in a slightly different way, then the results are combined (generally averaged) to give the final output. In a random forest the base learners are decision trees. Decision trees attempt to partition the feature space one variable at a time in the way that best classifies the data (i.e. the input variables are divided such that values above a point go into one class and values below a point go into a different class). This partitioning (splitting) of the input variables continues until no more splits can be performed or some stopping criteria are reached. To create a random forest, many decision trees were trained with each tree only seeing a random subset of the data, and at each split a random subsample of the input variables was tested for partitioning. Finally, all the trees were averaged to generate output probabilities. The random forest was implemented using the R package "randomForest" (Liaw and Wiener 2002).

Method	Summary	Advantages	Disadvantages
Penalised logistic regression	Logistic regression estimates the probability of an observation belonging to a class by transforming linear combinations of summary statistics. A penalty is added to the model for complexity which arises from multicollinearity of the summary statistics that causes models to overfit.	Less likely to overfit Short training time No parameter tuning Performs well with small N	Reliance on linearity Less accuracy
Support	SVM's use hyperplanes (decision	High accuracy	Resource intensive
vector	boundaries that defines what group an	Maximum margin	Over-fitting
machine	observation belongs) to group data into classes as defined by the user. The goal is to maximise the difference between the hyperplane and the closest elements of the respective classes. The maximum margin maximises the differences between classes, increasing confidence in classifications.	(reducing misclassification) Kernels are used to transform non-linear data into a new space where a linear function can separate them.	Difficult to interpret Sensitive to input parameters
Random	Random forests are created by making a	Variable importance	Does not perform
forest	series of decision trees and finding the mode or mean prediction of the trees in the forest. A decision tree uses sets of summary statistics to make decisions at nodes and the terminal node defines what class an observation belongs to.	estimates Efficient with large datasets (especially on multi-core machines) Very little pre- processing required Handles missing data Resistant to outliers	well with small N Searches only a small subset of the data for the best predictor to split on
Stochastic	Stochastic gradient boosting extends the	Non-linearity	Large memory
gradient	random forest model where new trees	Variable importance	footprint
boosting	are fitted to the residuals of the last tree.	Efficient with large	Fine scale tuning of
	And instead of using the mean of the models	datasets Very little pre	parameters required
	are added together.	processing required	Call Overni
		Handles missing data	
		Performs an	
		exhaustive search of	
		the best predictor to	
		split on	

Table 4.2. Summary, advantages and disadvantages of the models used to classify behaviours.

4.2.4.4. Stochastic gradient boosting

Stochastic gradient boosting machines (GBMs) are another form of ensemble learning (Friedman 2002). Although base learners can be in many forms, we implemented tree learners as the base learners. GBMs pre-form classification in an iterative fashion. In the first iteration a learner is trained to classify the problem. In each successive iteration another base learner is trained to explain the error from the previous iteration. Thus a GBM successively learns to explain the error of all previous iterations. Iterations continue until a stopping criterion is reached, generally the maximum number of iterations. GBMs are stochastic in nature due to each iteration is only shown a randomly selected subset of the data and at each stage in the tree building process only a random subset of the input variables is assessed for

splitting. To generate output probabilities all of the trees were averaged. The GBM was implemented using the R package "xgboost" (Ridgeway 2015).

All models were run in R (version 3.2.1) through the package "caret" (Kuhn 2016).

4.2.5. Training and testing

The data classes were imbalanced, therefore the effects of both under and over-sampling were tested and the resulting model performance assessed. Over-sampling can cause the model to over-fit, whereas under-sampling may lose vital information (Zughrat *et al.* 2014). Initial testing showed that undersampling performed slightly better than over-sampling, therefore under-sampling was used for the rest of the testing. Moreover, due to the large amount of data under-sampling was used with little restriction. We chose a class maximum to be 3000, smaller than the minority class size of 4084. Under-sampling was only used for the training data. Test data were left unchanged as this was more representative of wild data that would not be evenly distributed among behaviour groups.

In order to assess the influence of the feature statistics on our models each model was run twice, once with the summary statistics and once with the feature and summary statistics. To find the best parameters of the models the data with ten seals were split into training and validation sets, which were 70% and 30% of the data respectively and run across a grid of parameters. The models were trained on the (70%) data split using 10-fold cross-validation. Model performance for the data is as an average of the out-of-fold accuracy, e.g. the model is trained on 9-folds and then tested on the 10th fold. This process was repeated 10 times, each time using a different fold as the out-of-sample data, until all folds had been used. The final model performance (reported here) was the accuracy on the 30% validation split from which we found the best parameters for each model. We used these parameters to train a model with all of the data from the ten seals and used it to classify the behaviours of the two seals that were so far unseen by any model. Thus the final cross-validation accuracy was assessed on data that the model had not seen during training and gave a true picture of model generalisation.

4.3. Results

Through coding more than 20 hours of video footage we classified 6181 bouts split between the 27 behaviours (Table 4.3). Bouts of behaviour were clearly identified from the tri-axial accelerometry data (Fig. 4.3). 1344 bouts of behaviour were classified as other because they were behaviours that would not be seen in the wild (i.e. moving in and out of the pool, being fed by the marine mammal keeper) and were excluded from the analysis. This included 30 bouts of behaviour classified as playing, and while this behaviour in the wild is an important indicator of development and condition (Harcourt 1991b, Harcourt 1991a) the sample size was too small to compare it to the other groups of behaviour.
Category	Behaviour	Number of bouts	Category	Behaviour	Number of bouts
Travelling (N=2844)	Walking	535	Resting	Lying	17
	Surface swimming	1128	(N=829)	Sitting	532
	Swimming	1003		Still	280
	Fast swimming	121	Grooming	Scratch	67
	Porpoising	57	(N=331)	Rubbing	9
Feeding	Chewing	308		Sailing	28
(N=1759)	Foraging	249		Jugging	19
	Thrash	303		Face rub	54
	Manipulation	779		Shake	39
	Hold and tear	120		Grooming	115

 Table 4.3. Number of bouts of behaviours classified and their associated categories. (See Table

 A-1 for a description of the behaviours and an example of the associated accelerometry output).

Using 13 epochs we had a total of 92516 input variables for the model. This consisted of 64642 training inputs and 24795 testing inputs from the two seals selected for cross-validation. The final average accuracy from the training set of data without feature data for the baseline model (penalised logistic regression) was 64.0%, with poor testing results (47.0%). From the training results without features random forests were the most accurate in predicting behaviours, classifying on average 75.1% of the behaviours accurately (Table A-2). However, the cross-validation accuracy for this model was poor (48.6%). This was followed by stochastic gradient boosting machines (GBM) with an average accuracy of 73.7%, with cross-validation accuracy of 62.0%. SVM's achieved between 64.2 and 72.6% accuracy, with cross-validation scores ranging from 48.0 to 64.0%. The kernel used for SVM's was important in determining final accuracy where linear kernels produced the lowest accuracies and polynomial kernels produced the highest accuracies overall (Table 4.4). Adding feature data to the models improved the training and testing accuracy of all models. Random forests and GBM achieved over 80% training accuracy, though GBM had better performance on cross-validation (65.0%) than random forests (54.0%). Despite having lower training accuracy than the GBM and random forest, the SVM with

polynomial, linear and radial kernels all had higher cross-validation accuracies. The polynomial kernel had the highest cross-validation accuracy of any model, classifying 72.0% of the data accurately.



Figure 4.3 Example of raw acceleration data for a series of behaviours. The * represents a fish capture in the water column.

Within the training models resting was most often classified accurately (83-89%), followed by grooming (71-94%) and foraging (59-75%). Travelling was the most difficult category to classify (32-71%) (Table 4.5). The confusion matrices for the cross-validation accuracies on the two seals left out revealed a very different story and model influenced the overall accuracy of each behaviour category. Travelling was still the hardest behaviour to classify (31-58%) and the models now found resting much harder to classify (41-75%). Foraging was classified with the highest accuracy now (60-85%) followed by grooming (62-76%).

4.4. Discussion

Accelerometers have been used to build ethograms in a range of species, generally being able to predict the correct classification of a class more than 90% of the time, however we argue that this may be a result of highly selective data input and choices made in the analysis. In this study, we trained machine learning models to recognise four distinct, biologically-relevant, categories of behaviour: travelling, resting, foraging and grooming. Models were then tested on two seals previously unseen by the models and were tested both with and without feature statistics describing some characteristic of the seal. The choice of machine learning algorithm contributed to the overall prediction accuracy and adding feature statistics to the model improved the overall training and testing accuracies. By training our models on ten seals and cross-validating on two left out we ensured the generalisability of our models, and that they were robust to individual differences.

Table 4.4 Training and testing accuracy, with the best training parameters for seven machine learning models. Cross-validation accuracy (%) from training (ten animals) and testing models (out-of-sample; two animals) of machine learning models run with and without feature statistics and the best parameters used for testing.

Model	Train Accuracy	Test Accuracy	Best parameters
Without features			
GBM	73.69	61.98	Eta = 0.01 ; max.depth = 5; nrounds = 5000; subsample = 0.7
RF	75.08	48.63	Mtry = 10; $ntree = 1400$, $nodesize = 1$
RLR	63.72	46.91	Param1 = 0.810 param2 = 0.0012
SVM Linear	64.22	48.00	Cost = 100
SVM Sigmoid	65.08	46.29	Gamma = 0.0001; coef0 = 0; cost = 100
SVM Radial	71.25	59.71	Gamma = 0.001; cost=100000
SVM Polynomial	72.58	63.94	Degree = 4; gamma = 0.01 ; coef 0 = 4; cost = 1
With features			
GBM	80.81	65.04	Eta = 0.01 ; max.depth = 4; nrounds = 5000; subsample = 0.8
RF	80.53	53.92	Mtry = 12; ntree = 1000 , nodesize = 3
RLR	71.33	64.63	Param1 = 0.10 param2 = 0.0018
SVM Linear	71.50	68.15	Cost = 10
SVM Sigmoid	70.31	55.46	Cost = 100; coef0 = 0; gamma = 0.0001
SVM Radial	79.03	68.87	Cost = 10000; gamma = 0.001
SVM Polynomial	78.83	72.01	Cost = 0.1; $coef0 = 4$; gamma = 0.01; degree = 4

4.4.1. Supervised machine learning

Machine learning algorithms have regularly been used to classify animal behaviour from accelerometry data, with varying levels of success (Gerencser *et al.* 2013, Nishizawa *et al.* 2013, Carroll *et al.* 2014). With a range of algorithms available and the wide array of problems to which they can be applied, it can be overwhelming to be able to select an appropriate method that will provide the greatest accuracy (Nathan *et al.* 2012). Rapidly developing technology has improved computing speed and the ease by which machine learning can be implemented. This affords researchers the opportunity to test and examine different methods for their data. Here we tested four supervised machine learning algorithms on accelerometry data collected from captive fur seals and sea lions to assess their ability to predict behavioural states. We found that SVM with a polynomial kernel was the most accurate in being able to

classify behaviours from testing data (previously unseen by the model), but that GBM and random forests produced the best training results.

Table 4.5 Confusion matrix (observed – rows vs. predicted – columns) for the cross-validation results with dummies from the gradient boosting machine (GBM), random forest (RF), logistic regression (LR) and support vector machine (SVM) models. Only the results from the best SVM - polynomial (poly) - are presented here.

GBM	Foraging	Grooming	Resting	Travelling	Sensitivity	Specificity
Foraging	5717	66	132	821	84.9%	88.3%
Grooming	42	180	10	59	61.9%	71.4%
Resting	363	66	1773	332	70.0%	70.2%
Travelling	2226	1111	5020	11397	57.7%	36.0%
RF	Foraging	Grooming	Resting	Travelling	Sensitivity	Specificity
Foraging	4836	661	257	982	71.8%	74.9%
Grooming	36	183	16	56	62.9%	61.9%
Resting	508	38	1830	158	72.2%	60.2%
Travelling	3996	3681	1037	6520	42.8%	43.7%
LR	Foraging	Grooming	Resting	Travelling	Sensitivity	Specificity
Foraging	5671	115	174	776	84.2%	80.3%
Grooming	14	202	21	54	69.4%	62.4%
Resting	441	47	1843	203	72.7%	60.6%
Travelling	3094	3024	806	8310	54.5%	35.9%
SVM poly	Foraging	Grooming	Resting	Travelling	Sensitivity	Specificity
Foraging	5856	123	62	695	86.9%	81.3%
Grooming	52	188	6	45	64.6%	62.5%
Resting	697	314	1040	483	41.0%	61.6%
Travelling	2596	1258	483	10772	71.3%	30.9%

In a study on the behavioural modes of griffon vultures (*Gyps fuluus*) five machine learning algorithms were evaluated with random forests being the best predictor of behaviour (Nathan *et al.* 2012). While random forests also performed well when evaluating training data in our comparison, GBM (which was not evaluated by Nathan *et al.* (2012)) improved the accuracy. However, SVM with a polynomial kernel had the highest rate of cross-validation classification accuracy. SVM's have been used successfully in other behaviour classification studies that used accelerometers (Martiskainen *et al.* 2009, Campbell *et al.* 2013, Diosdado *et al.* 2015). It is likely that the best classification algorithm will differ for each data set and the behaviour type that is to be predicted. We found that different machine learning algorithms gave better results depending on whether it was training or testing the data. They also differed in the accuracies assigned to different behaviour categories.

Given the large variety of machine learning algorithms available and the relative ease of implementation and testing, we recommend evaluating a range of different algorithms to determine which gives the best performance for a particular problem. However, it is worth considering the training and testing times of the different models. For our example the SVM with polynomial kernel took \sim 2 days to run and the GBM took less than 2 hours on a dual 12 thread computed with 3.5 GHz processors. Therefore, it is worth considering if there are gains to be made in adjusting the model inputs as well as changing the model itself.

4.4.2. Groups of behaviours

We classified 27 behavioural states (Table A-1), one of which (playing), was not used as it occurred infrequently. This was too many groups for a model to classify realistically in terms of computational time and power. It also required a large investment of observer time to collect a large enough sample for each of the classes represented in the model. This is because an important step in the process is to ensure each behaviour or class is equally represented in the model. Rather than losing the detailed information of each of the observed behaviours, we grouped behaviours into states (e.g. Hammond *et al.* 2016). This technique can be useful in developing activity budgets for large data sets, particularly where one state dominates behaviour (e.g. swimming). This method may also prove useful in wild applications that aim to automatically classify the state of the animal in real time, before uploading a wireless data summary to a nearby receiver. Summarised data from accelerometers via wireless devices have been successfully used for monitoring human behaviours (Tapia *et al.* 2004), in particular for monitoring health conditions (Beniczky *et al.* 2013, Dewancker *et al.* 2014), but have not as yet been used for monitoring wild animals. This advance in technology has the potential to increase the efficiency and the data storage capacity of devices on wild tagged animals.

The four categories we created for this analysis (grooming, resting, travelling, foraging), represent the typical behaviours that would be used by these species in the wild (Stirling 1970, Marlow 1975). Resting had fewest cases of misclassification in the training stage as there was minimal movement on any axis and was consequently easy to predict. However, in the testing stage the prediction accuracy of resting, while still reasonable dropped 10-30% depending on the model. The models predicted grooming with reasonable accuracy in both training and testing which was probably because using a relatively short epoch allowed more active behaviours to be distinguishable from immobile behaviours (Halsey *et al.* 2009a). Travelling was predicted with the least accuracy in training and testing. Travelling was most commonly mistaken for foraging, which was not surprising considering the behaviours frequently overlapped. Foraging was predicted well, likely at the detriment of travelling. Usually, foraging behaviours are the most difficult to distinguish, particularly when they are of very short duration (such as a fish capture here or attack/peck in the plover (Bom *et al.* 2014)). Having a very short epoch likely allowed these behaviours to become more distinguished, while travelling behaviours became nosier. Repetitive behaviours perform better with longer epochs as the model could more readily find patterns

in the data (Diosdado *et al.* 2015). Therefore, using a longer epoch will likely strengthen the models ability to predict resting and travelling, but will reduce the accuracy of grooming and foraging.

The different kinematics of the behaviours likely influenced the ability of the models to distinguish between the classes. Resting behaviours were most related in terms of their kinematics as they represented prolonged periods of non-movement. The key feature that distinguished the resting behaviours from one-another was the bodies angle, which would be represented by a change in value of the x, y or z axis that would then remain static (Table A-1). Static behaviours are usually much more distinguishable from non-static behaviours (Yang et al. 2008), but as we have demonstrated here this is only over long epochs. Foraging and travelling were most confused by the model. Foraging behaviours are commonly long, repetitive behaviours interspersed with short, sharp and fast reactions (i.e. a head strike to capture a fish while swimming or thrashing a fish to tear it apart). However, foraging also consists of prey manipulation which often involved slow elongated movements of the body to change position and take control of the prey better, which could easily be interpreted as travelling. Travelling behaviours were predominantly repetitive swaying movements of the body, where peaks in acceleration corresponded to a flipper stroke or a step. Porpoising also reflected this pattern, though the peaks were larger and corresponded to returning to the water. Similar to resting, while these behaviours would be captured over long epochs due to the repetition, the shorter epoch causes the behaviour to get confused (most often as foraging). Grooming behaviours are predominantly continuous, short, sharp movement, which would be easily distinguishable from other behaviours. Grooming for seals also involves two static behaviours that could only be distinguished from resting due to the body angle, as sailing requires the animal to lie on its side, while jugging means the seal is upside down. There are some obvious categories of behaviour fundamental to the ecology of fur seals and sea lions that we were unable to capture. Play behaviour is an indicator of developmental stage and also a subtle indicator of changes in condition (Harcourt 1991b, Harcourt 1991a), but we had insufficient samples for analysis. Mating and social behaviours are largely absent from the accelerometry literature (Brown et al. 2013), and here we were unable to fill this gap as we did not record the animals mating. Because it is inherently difficult to observe mating behaviour, accelerometers have only been used for identifying reproductive behaviour of freeliving animals in a few instances (Whitney et al. 2010). Other behaviours that we did not observe but are known to be important in otariid ecology include regurgitation and vocalisations (Insley et al. 2003). The absence of these behaviours from this ethogram means that when these behaviours are captured in the wild, the learning algorithm will classify these as one of the pre-determined categories on which we have trained the model. When monitoring an animal over an extended period it can result in a misrepresentation of how animals spend their time.

4.4.3. Leave-two-out validation methodology

A goal of this study is to generate a robust model that can be used to predict the behaviour of wild seals, so it is essential that the model can be applied across a range of individuals. We tested this by training the model with data from ten seals and then testing the model on two seals previously unseen by the model. While the cross-validation accuracy was lower than the training accuracy, we were still able to classify the seals behaviour well with some of the models. Previously, the effect of individual has been shown to have a large influence on the overall accuracy of the model (Diosdado *et al.* 2015). Fitting a model to an individual generally causes it to over-fit, thereby losing the generalisability of the model. By including many different animals of different sizes, and testing it on two animals previously unseen by the model, we could use the best model to predict the behaviours of many otariids. However, it is uncertain whether this model could be used with other pinniped species. For example, the very different gaits of the phocids in water and on land would likely influence the overall predictive ability of the model (Pierce *et al.* 2011).

4.4.4. Influence of feature statistics (characteristics)

We chose characteristics that could easily be determined from animals tagged in the wild to test how they would influence the overall accuracy of the models. We found that by including these variables (place, age, sex, species, mass and accelerometer attachment method) the models' training and testing accuracies improved. The individuals in this study differed in age, sex, species and mass, which we hypothesised to influence model accuracy. Previously it has been shown that with dogs there were no differences behaviour prediction in inter-breed comparisons, and the lack of difference in body morphology probably explained the lack of difference (Gerencser et al. 2013). Here we suggest that including these types of information in the model can help improve accuracies. Sea lions as a class differ from fur seals in several aspects of body locomotion, and allowing the model to distinguish between the two might explain some of the model improvement (Beentjes 1990). It may also be explained by differences in prey processing tactics that we observed the species using (Hocking 2016), as this type of behaviour was not examined in the dogs. Specifically, sea lions can process prey with their fore-flippers and chew their food, a phenomenon not observed in fur seals (Hocking 2016). By including these details in the model we were able to improve training accuracy by between 5.3 and 7.8% and cross-validation accuracy by between 5.3 and 20.1%. Considering we would know these characteristics of wild seals it is a worthwhile endeavour to include these features in models.

4.4.5. Conclusions

The aim of this research was to determine the optimum method of automatically classifying many behaviours of a highly dynamic animal living in a complex environment using an accelerometer. Due to the large number of behaviours that animals can display, we further sought to investigate whether behaviours could be grouped for simpler prediction. Classifying behaviours of an animal is extremely difficult, and despite having captive animals under command we were still unable to capture all behaviours. Of the behaviours we did capture, only three of the groups of behaviours were able to be classified with good (>70%) accuracy (travelling had poor accuracy results).

These results are important for the application of accelerometers to wild animals. When using supervised machine learning to classify behaviour it is likely that the animal will display behaviours that have not

been trained into the algorithm. Therefore, the model will do its best to fit it into a group that fits the best. For models that have been trained on a few select behaviours, this means there will be a significant amount of time that the animals mode of activity will be misclassified, leading to inaccurate activity budgets (if that is indeed the goal of the research). For example, the poor result for classifying travelling in our study means that for around half the time that the seal is travelling, they will likely be classified as grooming or foraging.

These models are complex and need to be treated as such. Providing a model with many repeats (hundreds if possible) of highly diverse behaviours in a related environment is vital to being able to use this technology and these models on wild animals. However, this still does not guarantee that the behaviours observed from captive animals will directly translate to their wild counterparts. The environment in which behaviours were observed (captivity) is different to the wild. Small pools, dead prey and human instruction may alter the way that animals display behaviour. In particular, we were unable to replicate prey chasing in captivity that would have helped to differentiate between travelling and foraging. Captive surrogates have been used successfully to train models with vultures (Nathan *et al.* 2012) and when developing models from the same species an over 90% accuracy rate can be obtained (Campbell *et al.* 2013)

Applications of this type of behavioural analysis include developing time-energy budgets of free living seals. To estimate energy expenditure in the field the durations of different activities are multiplied by their corresponding energetic cost (Goldstein 1988). Ethograms developed from accelerometers provide the essential information of time spent in various activities, and using accelerometers energy expenditure can be estimated concurrently (Halsey *et al.* 2009a). Further, these types of models can be used to monitor populations of animals over time. For example, knowing how much time animals spend foraging between years can be indicative of the prey availability and can identify the potential vulnerability within groups (Boyd 1999).

Chapter 5

Super machine learning: improving accuracy and reducing variance of behaviour classification from accelerometry

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Abstract

Semi-automating the analyses of accelerometry data make it possible to synthesize large data sets. However, when constructing activity budgets from accelerometry data there are many methods to extract, analyse and report data and results. For instance, machine learning is a robust approach to classifying data. We used a new method, super learning, that combines base learners (different machine learning methods) in an optimal manner to achieve overall improved accuracy. Other facets of machine learning that can be altered include the number of behavioural categories to predict, the number of epochs (sample window size) used to split data for training and testing and the parameters on which to train the models. The super learner accurately classified behaviour categories with higher accuracy and lower variance than comparative models. The number of categories used significantly altered the overall accuracy of the models, wherein four behaviours classified with greater accuracy than six behaviours across all models. The number of epochs chosen also affected the accuracy with smaller epochs (7 and 13) performing better than longer epochs (25 and 75). Correct model selection, training and testing is imperative to creating reliable and valid classification models. To do so means model fitting must use a wide array of selection criteria. We evaluated a number of these including; model, number of behaviours to classify and epoch length and then used a parameter grid search to implement the models. We found that all criteria tested contributed to the models' overall accuracy. Fewer behaviour categories and shorter

epoch length improved the performance of all models tested. The super learner classified behaviours with higher accuracy and lower variance than other models tested. However, when using this model, users need to consider the additional human and computational time required for implementation. Machine learning is a powerful method for classifying the behaviour of animals from accelerometers. Care and consideration of the modelling parameters evaluated in this study are essential when using this type of statistical analysis.

Keywords

Behavioural classification, marine mammal, ethogram, accelerometer, machine learning, super learner

5.1. Introduction

Otariid pinnipeds, fur seals and sea lions, play an important role in the trophic interactions of many marine ecosystems, especially in the sub-polar and temperate seas of the southern hemisphere (Bowen 1997). Multiple species of otariid can be sympatric, driving complex interactions such as niche separation, where species specialize on different types of prey or segregate by feeding area (e.g. feeding in deep water at the sea floor vs. feeding near the surface in the open ocean; Jeglinski *et al.* 2013). This results in differences in behaviour and activity patterns among species that may be an important influence on their broader ecological community (Hindell *et al.* 2003). Despite the importance of this group to understanding marine ecosystems, there is still much to learn about the behaviour of these and other marine predators (Hays *et al.* 2016). This is primarily because it is very difficult to study marine animals in the wild as they are active in remote locations and deep underwater where direct observation is often not possible (Bowen *et al.* 2002, Hussey *et al.* 2015).

Advances in bio-logging technologies and computing power have enabled biologists to pry into the daily existence of many difficult to observe animals (Wilson *et al.* 2008, Hussey *et al.* 2015). A powerful approach is to create ethograms from accelerometers using machine learning. Accelerometers measure the inertial acceleration of an animal while moving, most commonly on three axes (Brown *et al.* 2013). Unique combinations of these three axes identify specific movements that correspond to a single behaviour or series of behaviours. Single behaviours can be identified with a high degree of accuracy; e.g. prey captures in penguins using support vector machines (SVM's) (Carroll *et al.* 2014). However, when trying to identify an entire ethogram of behaviour and the number of behavioural categories to be predicted influence the overall predictive capabilities of machine learning methods (Bom *et al.* 2014, Diosdado *et al.* 2015, Hammond *et al.* 2016). For example the attack/peck category in crab plovers cannot be predicted using decision trees, which may be a result of trying to classify too many categories (Bom *et al.* 2014). Hidden semi-Markov models predicted two categories of behaviours with much higher accuracy than three, four or five categories (Hammond *et al.* 2016). While longer window sizes used to

classify cow behaviour improved the overall accuracy of four machine learning methods tested (Diosdado et al. 2015).

The machine learning method selected to classify the data will also influence the overall accuracy (Nathan *et al.* 2012, Hammond *et al.* 2016). There have been several attempts to evaluate the accuracies of different machine learning methods (Nathan *et al.* 2012, Diosdado *et al.* 2015, Dutta *et al.* 2015). However, due to vastly distinct dynamic movement of different animal species, it is unlikely that there will ever be a universal set template for creating ethograms from accelerometry (Campbell *et al.* 2013). Instead, a new machine learning method described here may afford a solution to the problem of method selection. Super learning takes a set of candidate learners (other machine learning methods), applies them to a dataset and chooses an optimal learner or combination of learners based on the resultant cross-validated risk (van der Laan *et al.* 2007). The super learner model (SL) seeks to find the optimal combination candidate learners such that it will perform as well or better than any of the learner inputs (Davies and van der Laan 2016). Super learning has previously been applied to large medical datasets in order to make survival predictions with considerable success (Pirracchio *et al.* 2015), but has until now not been evaluated for its ability to classify behaviour from accelerometry data.

The ability to reliably build highly generalizable models for the classification of animal behaviour will be a significant advance for the study of those species that are difficult or impossible to observe in the wild or sustain in captivity (Campbell *et al.* 2013, Bidder *et al.* 2014). To reliably classify animal behaviours from accelerometry it is necessary to evaluate the performance of different models and their parameters (Nathan *et al.* 2012). The aims of this study are twofold: 1) assess by how much super learning can improve the accuracy of classifying accelerometry data in general and 2) identify the optimal time window and number of behaviour categories required to create reliable ethograms for fur seals and sea lions with different foraging specialisations.

5.2. Materials and Methods

5.2.1. Animals

We conducted captive experiments at three Australian marine facilities: Dolphin Marine Magic, Coffs Harbour (RF1: 30°17'S, 153°8'E); Underwater World, Sunshine Coast (RF2: 25°40'S, 153°7'E); and Taronga Zoo, Sydney (RF3: 33°50'S, 151°14'E) from August to November 2014 and again at RF2 in August 2015. We used two Australian fur seals (*Arctocephalus pusillus doriferus*), three New Zealand fur seals (*Arctocephalus forsteri*), one subantarctic fur seal (*Arctocephalus tropicalis*) and six Australian sea lions (*Neophoca cinerea*) (Table 5.1). All seals were on permanent display at their respective marine facilities and were fed and cared for under the guidelines of the individual facility. All Australian sea lions in the study were born as part of an ongoing captive breeding program in Australian aquaria. All fur seals came into captivity as juveniles after they were found in poor health or were injured and were deemed unsuitable for release back into the wild.

5.2.2. Experimental protocol

We used a tri-axial accelerometer (CEFAS G6a+: 40mm x 28 mm x 16.3 mm, 18 g in air and 4.3 g in seawater, CEFAS technology Ltd, Lowestoft, UK) to measure the movement of the seals. We used two attachment methods for accelerometers: either taped between the shoulder blades, or secured in a custom designed harness. Accelerometers were set to record at +-8g and at 25 samples per second (25Hz). We recorded all trials continuously with one or two cameras (GoPro Hero 3 – Black edition, USA; HDRSR11E: Sony, Japan), and trials had a maximum duration of 2.5 hours. Videos were scored to an ethogram consisting of 26 unique behaviours developed previously (Chapter 4). We time-matched the videos and the accelerometry output to generate annotated acceleration datasets.

Table 5.1. Study species and characteristics. Seal identification, marine facility, species, age, mass range, sex, number of trials and method of accelerometer attachment for fur seals and sea lions used in the study.

Seal ID	Marine	Species	Age	Mass	range	Sex	# of trials	Attachment
	facility			(kg)				method
ASF1	RF1	ASL	5		44-47	Female	13	Harness
ASF3	RF2	ASL	17		58-74	Female	4	Harness
ASF4	RF1	ASL	17		66-70	Female	12	Harness
ASF6	RF1	ASL	7		50	Female	2	Harness
ASM1	RF1	ASL	9	10	08-110	Male	8	Harness
AFF1	RF2	AFS	17		69-79	Female	7	Tape
AFM1	RF2	AFS	16	17	75-242	Male	7	Tape
ASM2	RF3	ASL	13	16	50-162	Male	9	Таре
NFM1	RF3	NZFS	8		47-54	Male	5	Таре
NFM2	RF2	NZFS	11	10)8-152	Male	5	Таре
NFM3	RF3	NZFS	13	11	1-154	Male	8	Tape
SFM1	RF2	SFS	4		28-30	Male	3	Таре

AFS - Australian fur seal; NZFS - New Zealand fur seal; SFS – subantarctic fur seal and ASL - Australian sea lion.

5.2.3. Behaviour segmenting

We grouped the 26 behaviours into broader behavioural categories. As the number of behavioural categories used to classify behaviour may affect the overall results we chose to test four (feeding, grooming, resting and travelling) and then six categories (feeding, foraging, thrashing, grooming, resting and travelling). We also compared the ability of the model to discriminate behaviours over a range of discrete periods. We tested four epochs (number of accelerometer samples): 7 (0.28 second), 13 (0.52 seconds), 25 (1 second) and 75 (3 seconds) (Alvarenga *et al.* 2016). Behaviours could also be

"contaminated" where two behaviours occur in the same time window. In these cases, we used the dominant behaviour with resultant windows of uneven time duration.

5.2.4. Summary statistics

We created 147 summary statistics as the inputs to the machine learning models. Summary statistics related to the animal or the behaviour included were: where the behaviour occurred (surface, underwater or land), device attachment method (harness or tape), age, mass, sex and species of the individual. For each of the three axes (x, y, z) we calculated mean, median, minimum, maximum, range, standard deviation, skewness, kurtosis, absolute value, inverse covariance and autocorrelation trend (the coefficient derived from a linear regression) and the 10th and 90th percentiles. We also calculated *q* as the square-root of the sum-of-squares of the three axis (Nathan *et al.* 2012), and included pair-wise correlations of the three axis (x-y, y-z, x-z) (Ravi *et al.* 2005). The inclination as azimuth were calculated as per Nathan et al. (2012). We calculated three values of dynamic body acceleration (DBA) by using a running mean of each axis over 3 seconds to create a value for static acceleration value to create a value for partial dynamic body acceleration (PDBA). We calculated overall dynamic body acceleration (ODBA) (Wilson *et al.* 2006, Shepard *et al.* 2008a) using;

$$ODBA = |X_{dyn}| + |Y_{dyn}| + |Z_{dyn}|$$
(5.1)

We calculated vectorial dynamic body acceleration (VeDBA) (Qasem et al. 2012) using;

$$VeDBA = \sqrt{X_{dyn}^2 + Y_{dyn}^2 + Z_{dyn}^2}$$
(5.2)

We calculated the area under the curve for both ODBA and VeDBA using the package "MESS" in R (Ekstrom 2014, R Core Development Team 2015). The minimum, maximum, and 10th and 90th percentiles were calculated for PDBA, ODBA and VeDBA.

5.2.5. Classification models

There are many candidate models suitable for classifying behavioural data obtained from accelerometry (Nathan *et al.* 2012) and choosing the most appropriate method for the data in question can be complicated and time consuming. The super learner model (SL) combines candidate models (other machine learning models, henceforth referred to as base learners) by applying a selection of them to a set of data and then weighting these learners through another learner. The optimal combination is chosen based on cross-validated risk (Sinisi *et al.* 2007, van der Laan *et al.* 2007). The base learners chosen for this study were: random forests (RF), gradient boosting machine (GBM), and a baseline model, logistic regression (LR) to which performances of the other models could be compared. Logistic regression was included as a baseline model as it is well tested, easy to implement and unlikely to overfit. RF and GBM have been shown to fit this type of data well previously and can be implemented with relative ease (Chapter 4; Nathan *et al.* 2012). Each base learner was trained across a set of parameters, with the

predictions of each model kept. These predictions, plus the raw data, then became the inputs to the SL. The SL then learned from the predictions of the base learners as well as the summary and feature statistics to predict the outcomes.

For each of the models data were split into a train (evaluation) and test (validation) set using 70% and 30% of the data respectively. Note that the test data were not seen by the model during training. This ensured that the scores obtained from the models reflected the ability of the model to predict from data outside training. Results of the model were reported as cross-validation scores and out-of-sample scores, which include accuracy and kappa. Cross validation is the process of breaking the data into smaller folds (usually 10) and training the data on most folds (usually 9) and testing the model on the fold left out. The summary statistics come from the average of the result of the tests of the fold that was left out of the training. Out-of-sample scores are those which results from testing how well trained models can predict group association of unseen data (data the model was not trained with). Accuracy was the proportion of true positives identified by the model while kappa was employed when two or more observers were used to classify data and accounted for the fact that some of their observations will agree or disagree by chance (Viera and Garrett 2005). This value was used to assess agreement of observed and predicted values in the confusion tables (Alvarenga *et al.* 2016).

5.2.6. Parameter grid search

Within each model there were a number of parameters on which models can be trained. Samples of each of these parameters were chosen and each model was run through every combination of these using a grid search within each model (Table 5.2; Table A-3). We evaluated best parameter grids of each model using H20 (Lendell 2015) for GBM and RF, glmnet (Friedman *et al.* 2010) for LR and the SL.

Table 5.2. Parameters for the four models tested. Nbins – number of bins; Mtry - number of splits
in branches; ntree - total number of trees grown; max depth - maximum depth to grow the trees (For a
detailed description of the model parameters and how they are used see Appendix 1).

Model	Nbins	mtry	ntree	max depth	
Random forest	20, 30, 40 (numeric)	5, 10, 15	200	5, 10, 15	
	5 (categorical)				
	Nbins	Learn rate	ntree	max depth	Sample rate
Gradient boosting	20, 30, 40 (numeric)	0.1, 0.001	250, 700	5, 10	0.7, 0.8, 0.9
machine	3 (categorical)				
	Lambda	alpha			
Logistic regression	range: exp(-11) to	0 to 1 by 0.025			
and super learner	exp(6)				

5.3. Results

Tri-axial acceleration data were collected from 12 seals over a range of trials lasting in duration from ten minutes to 2.5 hours (Table 5.1). From these we were able to mark 7525 bouts of behaviour, split into either four or six categories (Table 5.3).

5.3.1. Comparing model performance

All three test models (SL, RF and GBM) had significantly higher accuracies across the range of epochs and categories of behaviour tested compared to the baseline model (LR; Fig. 5.1). SL classified categories of behaviour with higher accuracy and lower variance than both RF and GBM across all epochs (except GBM 7 epochs, 6 categories). The variance was reduced by ~70% across all model combinations tested and accuracy was improved by between -0.1 and 10.1% (Fig. 5.1; Table A-3). The variances obtained from the logistic regression models were similar to the SL. Accuracy and precision of all models improved when using four as opposed to six categories of behaviour. Looking at the overall performance of the models from the highest cross-validation score, out-of-sample score and the kappa score, we concluded that using 13 epochs produced the best results across the four models (Table A-3).

Categ	gories	Categories							
Four	Six	Behaviour	N bouts	Four	Six	Behaviour	N bouts		
		Walking	545			Chewing	309		
		Surface swimming	1133	143	[4]	Manipulation	792		
[1] Travelling (N=2864)	1]	Swimming	1008	[4] Feeding	Feeding $(N=1615)$	Capture	394		
	elling 2864)	Fast	121	(N=1841)	(11 1010)	Hold and tear	120		
		Porpoising	57		[5] Foraging (N=226)	Searching	226		
[2] Resting		Lying	17						
		Sitting	541	[6] Thrashi	ng (N=303)	Thrashing	303		
(N=	839)	Still	281						
		Scratch	68			Playing	30		
		Rubbing	10	Other $(N = 1344)$		In/Out	475		
	[3]	Sailing	29	(1N - 1344)		Other	839		
[3]	(N=245)	Jugging	19						
Grooming $(N=334)$	(11 - 10)	Face rub	54						
(1 1 -33 4)		Rolling	115						
	[NA] High frequency	Shake	39						

Table 5.1 Number of unique behaviours observed for each category of behaviour.

5.3.2. Identifying categories of behaviour

Across all models and epochs, grooming and resting classified with the highest accuracy, with grooming generally outperforming resting (Fig. 5.2; Table A-4). Examining the confusion matrix from the best performing model (SL – four behaviours, 13 epochs) the classification errors from the four categories of behaviour revealed that foraging often misclassified as travelling and vice-versa (Table 5.4). Overall, within the test models (SL, RF, GBM) all four behaviours were correctly classified more than 75% of the time (Fig. 5.2). Within the six behaviour categories, the main misclassification stemmed from feeding, where only the super learner classified it correctly more than 50% of the time. The "thrashing" category that was also added to the model was classified with high accuracy (>75%). Resting and grooming maintained their high predictive accuracies across the test models (>80%). Foraging also maintained a reasonably high rate of classification (>70%), while traveling lost around 10% accuracy when compared with the four behaviour models.

	Foraging	Grooming	Resting	Travelling	Precision	Sensitivity
	Super Lea	rner				
Foraging	1248	17	53	182	0.83	0.82
Grooming	18	1292	27	64	0.92	0.91
Resting	80	37	1321	61	0.88	0.89
Travelling	185	79	77	1158	0.77	0.79
	Gradient I	Boosting Mac	hine			
Foraging	1243	23	54	180	0.83	0.81
Grooming	20	1300	30	52	0.93	0.89
Resting	80	39	1305	76	0.87	0.90
Travelling	191	92	68	1149	0.77	0.79
	Random F	orest				
Foraging	1220	25	57	198	0.81	0.80
Grooming	17	1291	42	52	0.92	0.90
Resting	86	35	1312	67	0.87	0.89
Travelling	195	88	59	1158	0.77	0.79

Table 5.2 Confusion matrices from three test models using four behaviours and 13 epochs.



Figure 5.1 Classification accuracy from cross- and out-of-sample validation of four different machine-learning algorithms. Coloured points (blue: four feature models; orange: six feature models) represent out-of-sample accuracy with error bars of ± 1 SD. Red bars represent cross-validation accuracy for each associated model.

5.4. Discussion

The aim of this study was to assess whether super learning (SL) would improve the predictive ability of base learners (RF, GBM and LR) to classify fur seal and sea lion behaviour from accelerometry. While building machine learning models, a number of choices must be considered about how to segment the data. We evaluated several combinations of time segmentation and number of behaviour categories for this type of accelerometry data to determine the most effective. Using super learning increased the accuracy of the models, albeit only slightly, and reduced the prediction error when compared with RF, GBM and the baseline model – LR. Shorter time windows (<13 samples) and fewer categories of behaviour (4 vs. 6) were better at predicting the behavioural state of the seal.



Figure 5.2 Classification accuracy of behaviour across epochs and models. We tested four (a) and six (b) categories of behaviour across four (SL, RF, GBM and LR) models across four (7, 13, 25, 75) epochs.

Four behavioural categories had a higher classification rate than six behaviours. At its most basic, accelerometers discriminate between two behavioural states (e.g. activity vs. resting or swimming vs. prey capture) and can do so accurately (Takahashi et al. 2009, Carroll et al. 2014). Adding more categories for the model to discriminate increases complexity, but reduces the uniqueness of the model, thus decreasing its overall accuracy (Diosdado et al. 2015, Hammond et al. 2016). There is also a greater chance of overlap with other behavioural categories. Increasing behaviour categories from four to six produced an overall average 11.5% (range: 9.5-14.5%) decrease in accuracy. The optimal number of categories becomes a trade-off between useful ecological information and high accuracy. Reducing the number of categories broadens the scope of the remaining categories as more similar behaviours are considered together and are thus easier to discriminate by the model. An important distinction to make is that considering fewer categories does not mean removing behaviours from the models, because if those behaviours are observed in the wild, the model will still try to classify them, resulting in an inaccurate representation of what the animal did while being monitored (for a discussion of this issue see (Ladds et al. 2016c)). As the loss in accuracy is so small, this leaves it up to the researcher to determine whether quality (fewer behaviours – more accuracy) or quantity (more behaviours – less accuracy) is important in the study. In this illustration of the method, which is broadly applicable to all free-living animals that can be equipped with accelerometers, we used fur seals and sea lions. For species such as these, four behavioural categories appear to be the minimum that provides meaningful information about their activities. In future studies that use this method, the number of categories must be tailored to the species concerned and aims of the study.

5.4.1. Epoch size – smaller is better?

We found that smaller epochs gave better overall predictions, and that the length of the epoch was significant in predicting different categories of behaviour. Increasing the window size reduces the sample size, which likely decreases the overall ability of the models to predict accurately. Having smaller epochs increases the sample size and reduces the chances of the model overfitting. Contrary to our results, a study of cow behaviour found that longer epochs tended to perform better than shorter epochs (5 and 10 min vs. 1 min) (Diosdado *et al.* 2015). While a similar study with humans discovered that epochs of one to two seconds had the best precision values (Huynh and Schiele 2005). Consistent with our findings, they also found that epoch length significantly affected the overall accuracy of individual behaviours.

We found different classification accuracies from the number of behavioural categories chosen, in this case from adding thrashing and feeding to the model. All models predicted thrashing with high accuracy (~75%), while only the SL predicted feeding with more than 50% accuracy. Thrashing is a very distinctive behaviour, with accelerometer readings exceeding 4g; very few other behaviours have this quality. By contrast, we defined feeding as a seal taking fish out of the water column, and animals were swimming while taking fish, therefore it was difficult for the models to distinguish between these two behaviours. Specific behaviours have been shown to be difficult to classify in other species. For example the

attack/peck category from crab plovers was unable to be classified better than chance. This stemmed from having too few examples of the behaviour to train the model to recognise it. Any additional behaviours added to the base four-category model need to be very distinct from any other behaviour and need enough examples to be representative in the model. Future studies investigating seal feeding behaviour should seek to gather examples of seals capturing live prey.

The differences arise from a combination of factors including epoch length, sample size, resolution, and behaviour category. For the scenario that we tested rapid behaviours were unable to be detected over long epochs as the behaviour becomes diluted by other behaviours, while for long duration behaviours, a long epoch was necessary to capture the features of the behaviour. This result is unique to this study because of the number and type of behaviours we selected, the frequency at which they were recorded at, and the epoch length chosen.

5.4.2. Super models – is it worth it?

The idea of a super machine learning model is enticing, allowing a multitude of machine learning models to be trained and tested on a single set of data thus allowing the model to optimally combine each of the individual models to give better overall predictions. Super learning has been successfully used in medical research (Pirracchio *et al.* 2015) and spatial analyses (Davies and van der Laan 2016), and improved the behaviour classification models from accelerometry, albeit marginally. With the exception of a single model combination (GBM; 7 epochs, 6 features), the super learner performed better than any other model combination. This was expected as super learning will use the optimal model it has trained on if it is unable to compute a more optimal solution (Davies and van der Laan 2016). We found an average increase of 3.4% (range -0.1-10.1%) in the classification accuracy of the models using super learning. While any improvement in model performance is welcome, single state of the art algorithms like GBM are easy to implement in software environments like R. Therefore, it is up to the individual researcher to decide if the additional human and computational time required to implement super learning is beneficial for their behavioural data study.

Chapter 6

Validating accelerometers to predict stroke rate using captive fur seals and sea lions

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Abstract

Energy expenditure of free-living fur seals and sea lions (otariids) is a vital currency to measure, but it is difficult to do so directly. An affordable, non-invasive, easy to interpret proxy for energy expenditure, such as measuring stroke rate, is a potential solution for this difficult task. Stroke rate is the driver of most mechanical output of otariids while at sea and can potentially be measured from small and costeffective accelerometers. However, estimating the actual number of strokes from an accelerometer depends on the processing of the raw data, and is yet to be validated for otariids. We videoed 10 captive otariids swimming underwater in two conditions; either wearing a harness with the accelerometer recording at 32Hz (N = 4) or with the accelerometer recording at 25Hz taped onto the fur (N = 6). We used 25 combinations of two parameters (running mean and gradient) to test the estimated stroke rate from the accelerometers by comparing these with the actual number of strokes counted from videos. We used a range of running means (0.4, 1, 2, 3 and 4 seconds) to smooth the acceleration data and five different gradients (the minimum number of consecutive positive data points) to detect a peak in acceleration that corresponded to a stroke. We tested all 25 combinations using the data from the x-axis, the z-axis and the x+z axes to determine if we could determine stroke rate from a single axis. We found that the running mean selected had little effect on the overall predictions, while using the x-axis only generally resulted in fewer errors. The performance of gradients was related to the rate of recording, where a higher gradient worked best for higher rates of recording. Over the range of parameters tested, total number of strokes were over-or under-estimated by up to $\sim 20\%$. We simulated the effect of different gradients (using a running mean of 3 seconds) to estimate stroke rate on the energy expended from a typical foraging trip of a female fur seal, and found that selecting a sub-optimal gradient (from the range of values tested here) would result in overall under- or over-estimation of energy expenditure of 7000-17500kJ over an entire foraging trip, which represented less than 1% of total energy typically

expended. When parameters are appropriately tuned, accelerometers are a simple yet valid tool for estimating the stroke rates of swimming otariids.

Key words

Otariid, swim mechanics, stroke rate, accelerometer

6.1. Introduction

Measuring the energy expenditure of free living animals is an important but difficult task. For free swimming pinnipeds, numerous proxies for rates of energy consumption have been tried and tested with mixed results, including heart rate (Boyd et al. 1999), doubly labelled water (DLW) (Jeanniard-du-Dot et al. 2016b) and overall dynamic body acceleration (ODBA) (Fahlman et al. 2013). Among the inherent difficulties in using these measures is the fact that the predictive equations for each are liable to change with species, size of the animal, activity type, temperature, and digestive state (Fish 2000, Ladds et al. 2016a, Rosen et al. 2016). Therefore, it is unlikely there will ever be a universal method, or universal equation, that can accurately estimate energy expenditure for pinnipeds as a group. However, proxies can still provide important comparative information and may have important practical application. Measuring stroke rate is a relatively non-invasive method that has been suggested as having high potential for predicting energy expenditure in both otariid seals e.g. Northern fur seals (Jeanniard-du-Dot et al. 2016c) and in phocid seals e.g. Weddell seals (Williams et al. 2004b). That stroke rate is a good predictor for these two species, which are evolutionarily divergent with completely different mechanics for underwater propulsion (otariids propel themselves using a sculling motion of their large fore-flippers (Feldkamp 1987), while phocids rely on lateral movement of their hind flippers (Gallon et al. 2007)) suggests stroke rate might have wide application as a proxy for energy expenditure (Williams et al. 2004b). Although stroke rate may seem a logical proxy for energy expenditure, there are questions regarding whether measures of stroke rate can be accurately obtained in free-swimming pinnipeds.

Pinnipeds perform long and deep dives traversing far-flung areas of the ocean while hunting and so are near impossible to observe *in situ* to count stroke rate. Animal borne cameras have been used to count strokes for some phocids seals, dolphins, and whales (Williams *et al.* 2000), but these devices are expensive, large and often fragile, with severe memory and battery limitations. While their use is insightful, simpler biotelemetry devices are much more widely deployed to provide insights on the location, physiology, and behaviour of seals at sea. Accelerometers are small, lightweight, and relatively cheap devices that measure the acceleration of the body on up to three axes, making them ideal for measuring stroke rate. Indeed, accelerometers have been successfully used to estimate stroke rates from a number of marine animals including penguins (Sato *et al.* 2011), seabirds (Lovvorn *et al.* 2004), fish (Broell *et al.* 2013) and sharks (Gleiss *et al.* 2009). They have also been used to estimate stroke rate for northern fur seals (*Callorhinus ursinus*) and Antarctic fur seals (*Arctocephalus gazella*) (Jeanniard-du-Dot *et al.* 2016c), though the predictions were not validated.

Estimating stroke frequency from accelerometers assumes that the peaks in the outputs correspond to a stroke. Phocids stroke with their rear flippers with a lateral sway, therefore it is assumed that peaks in the sway axis (y axis) correspond to a stroke (Williams *et al.* 2004b). Otariids use large fore-flippers to propel forward, therefore peaks in the surge (x axis), heave (z axis) or both axes are used to estimate strokes (Jeanniard-du-Dot *et al.* 2016c). Peak in lateral sway has been confirmed for Weddell seals using animal-borne cameras (Williams *et al.* 2004b), but there is yet to be a validation study for otariids.

The task of delineating strokes from accelerometry data is highly dependent upon the processing of the raw data. For example, the choice of running mean influences the overall estimate (Shepard *et al.* 2008a). Estimating strokes from accelerometers requires appropriate smoothing of data and the choice of a minimum gradient to determine a peak in the data. The choice of these two parameters will influence the overall estimate and so should be tested across a range of values. Here, we evaluated the best parameters for calculating stroke rate for otariids swimming underwater using animals observed and filmed in aquariums while swimming and wearing accelerometers.

6.2. Materials and Methods

6.2.1. Animals

We conducted experiments between October and December 2014 at two research facilities: Underwater World (RF2: Mooloolaba, QLD, Australia) and Taronga Zoo (RF3: Sydney, NSW, Australia) with three New Zealand fur seals (Arctocephalus forsteri), two Australian fur seals (Arctocephalus pusillus) and one Australian sea lion (Neophoca cinerea) that were on permanent display at their respective marine facilities. We conducted experiments in November and December 2015 at the University of British Columbia's Open Water Research Station (RF4: Port Moody, BC, Canada) with four Steller sea lions (Eumetopias jubatus) housed for research purposes (see Table 6.1 for details of the animals). All animals were nonreproductive during the study period and were cared for under the husbandry guidelines of the individual facility. All animals were in good health and condition as assessed by the in-house veterinary surgeon at the time of the experiments. All Steller sea lions were collected from breeding rookeries as pups and raised at the Vancouver Aquarium (British Columbia, Canada). Macquarie University ethics committee $(ARA-2012_064)$ and Taronga ethics committee (4c/10/13) approved experiments conducted in Australia. All animal handling and experimental procedures in Canada were conducted in accordance with regulations of the Canadian Council on Animal Care (University of British Columbia animal use permit #A11-0397), Department of Fisheries and Oceans Canada (MML 2007-001) and the Vancouver Aquarium.

6.2.2. Trial protocol

During all experiments otariids were equipped with a 3-axis accelerometer (RF1-3: CEFAS G6a+, \pm 8g, 40 × 28 × 16.3 mm and mass 18 g in air and 4.3 g in seawater, CEFAS technology Ltd, Lowestoft, UK; or a RF4: Daily Diary, 95 × 45 × 26 mm, 90 g, Wildlife Computers; Table 6.1) They were also recorded

with an array of static underwater cameras (GoPro Hero 3 Black edition, 1080p / Wide / 60fps). Seals from RF2-3 had the accelerometer attached between their shoulder blades with Tesa tape. Sea lions at RF4 had been trained to swim underwater between two submerged feeding stations (Rosen *et al.* 2016) while otariids at RF2-3 were trained to swim laps of a pool between two stationary targets. All animals were familiar with the experimental equipment and performed all trials voluntarily under trainer control.

Table 6.1 Seal characteristics. Species, ID, mass (kg), age (years) and marine facility where housed, type of accelerometer used, recording rate and method of attachment for five fur seals and eight sea lions. Marine facility: RF2 – Underwater World; RF3 – Taronga Zoo; RF4 – Open Water Research Station.

Species				Marine		Recording	Attachment	
Species	ID	Mass	Age	Age facility Device		rate	method	
Australian	AFF1	69-78	17	RF2	G6a+	25Hz	Таре	
fur seal	AFM1	179-182	14	RF2	G6a+	25Hz	Tape	
Australian sea lion	ASM1	153-160	160 12 R		RF3 G6a+		Таре	
New	NFM1	54-55	8	RF3	G6a+	25Hz	Таре	
Zealand	NFM2	149-161	11	RF2	G6a+	25Hz	Tape	
lui scai	NFM3	154	13	RF3	G6a+	25Hz	Таре	
Steller sea	F00BO	155-160	15	RF4	Daily Diary	32Hz	Harness	
lion	F97HA	172-175	18	RF4	Daily Diary	32Hz	Harness	
	F97SI	230-233	18	RF4	Daily Diary	32Hz	Harness	
	F00YA	214-218	15	RF4	Daily Diary	32Hz	Harness	



Figure 6.1 Dynamic acceleration of the x axis from a running mean of 1 second with different gradients applied to find peaks. A – gradient = 10; B - gradient = 20; C - gradient = 30; D - gradient = 40. Actual number of strokes for this sample (as determined by video footage) was 26.

6.2.3. Stroke rate estimation

Accelerometers (described above) recorded time, depth, and acceleration on 3 axes: anterior-posterior (surge), lateral (sway) and dorso-ventral (heave), from which the stroke rate, were extracted (see below). Video footage from RF2, RF3 and RF4 was pseudo-randomly collected with 10 animals participating in other experiments (Ladds *et al.* 2016a) from which we could directly count stroke rate. Underwater swimming at RF2 and RF3 was recorded with GoPro HERO3 (GoPro, USA) mounted inside PVC pipes each with a viewing window cut-out that were placed in the pool during trials (see Hocking *et al.* 2015 for a figure of the set up). Sea lions at RF4 had the GoPro mounted to their harness and oriented towards

the pectoral flipper. Videos were downloaded and edited together in Adobe Premiere Pro (Adobe Systems Incorporated, California), before being exported at the same frame rate as the accelerometer recorded (i.e., G6a+ 25 FPS and 25Hz at RF1-3; Daily Diary 32 FPS and 32 Hz at RF4). Accelerometer data were matched with the corresponding frame rate on the video, allowing us to extract data for dives.

The dynamic acceleration was then used to predict stroke rate, where strokes were identified as peaks in the x-axis (see Fig. 6.1, and description below). Actual total stroke number for a trial was counted from videos of individual trials, where a stroke was counted if a complete cycle of movement of the flipper was completed. Strokes that used a single flipper or that were only below the body were not included as they were often masked on the accelerometry by other movement. The accelerometry data is first smoothed using a running mean to remove the effect of gravity from the data. To detect a stroke a suitable gradient must be selected to detect a peak in the smoothed accelerometry. A peak is detected by calculating the signed difference iteratively of the smoothed data, then assigns a peak if the data are positive for the minimum amount of time set by the gradient. For example, if the minimum gradient is 10, then if there are at least 10 consecutive positive differences a peak will be marked at the end of the run of positive numbers (before the next negative number). The running mean used and the gradient of the peak affected the overall ability of the peaks to predict total stroke rate, therefore a combination of these variables was created and tested for their ability to predict total strokes for a dive.

6.2.4. Statistical analysis

As the recording rate and the attachment method of the accelerometers differed for some of the animals the analysis was carried out for two groups – the tape group (recording at 25Hz and accelerometer was taped to the fur) and the harness group (recording at 32Hz and the accelerometer was fitted to a harness). Stroke rate was estimated from peaks in the dynamic acceleration of the x, z and x + z axes. Peaks were extracted from the accelerometry data based on a minimum gradient before a peak. Since both the running mean and the minimum gradient of the peaks influenced the overall stroke rate estimate, combinations of these two variables were created. The running means tested were 0.4, 1, 2, 3 and 4 seconds and the gradients tested were 10, 20, 30, 40 and 50 (Fig. 6.1) for the tape group and 60, 70, 80, 90 and 100 for the harness group. The gradients differed for the two groups as the accelerometers were recording at different Hz; higher Hz resulted in more data being recorded which required a higher gradient. The best stroke rate prediction was defined as the running mean and gradient that resulted in the fewest errors when compared to observed stroke rates. This was determined by testing if the differences were significantly different from 0, meaning no difference in observed and measured strokes, using a one-sample Z-test.

To assess the impact of the different stroke estimates we investigated how they would affect overall energy expenditure estimation. The expected energy expended from each dive was calculated for both groups by multiplying the total number of actual strokes and estimated stroke rates for that dive by 7.9 J/kg/stroke. We chose this value as it was the estimated cost of a stroke similarly sized Southern sea lions (*Otaria flavescens*) (Dassis *et al.* 2012). As this was an exercise in the utility of using stroke rate as a

measure of energy expenditure we did not test all combinations of running mean and gradients. Instead for the estimated stroke rates we used a running mean of three seconds (as this is a commonly used smoother in the literature) and a range of gradients: 20,30, 40 and 50 for the tape group and 60, 70, 80 and 90 for the harness group. For each dive, the estimated energy expended for the actual number of strokes was subtracted from the estimated energy expended for the estimated stroke rate. The difference in energy expenditure was then plotted on histograms for comparison.

All analysis was completed in R (Version 3.1.3; R Core Development Team 2015) and values are reported as mean \pm SD.

Table 6.2 Summary of the combination of running mean and gradient that best predicts stroke rate for five fur seals and five sea lions. With the mean of the difference and the percentage difference between the predicted and actual number of strokes for that combination and number of trials.

ID	Method	Ν	Best	t runni	ng	Best			Mea	n		Diffe	rence	
			mea	n (secs	5)	gradie	ent		diffe	rence		%		
			Х	Ζ	XZ	Х	Ζ	XZ	Х	Ζ	XZ	Х	Ζ	XZ
Australia	n fur seal													
AFF1	Tape	12	4	0.4	0.4	40	40	40	0.2	0.1	0.6	-3.7	0.5	1.9
AFM1	Таре	19	0.4	0.4	0.4	50	50	50	3.1	2.3	2.6	-7.1	-5.7	-6.7
New Zea	land fur se	al												
NFM1	Таре	12	0.4	4	4	40	50	50	0.5	0.2	0.1	-2.1	-1.3	-1.5
NFM2	Таре	19	0.4	1	1	50	50	50	2.7	0.4	1.5	-2.2	-1.0	-2.6
NFM3	Таре	7	1	1	0.4	40	40	40	0.0	0.4	0.0	-0.3	-5.8	-2.6
Australia	n sea lion													
ASM1	Таре	17	4	4	3	30	40	40	2.2	0.4	1.0	-5.0	-2.8	-1.6
Steller sea	a lion													
F00BO	Harness	14	3	4	2	90	90	80	0.1	0.1	0.2	-1.0	-0.8	-1.6
F97HA	Harness	7	2	4	4	60	70	70	1.0	0.9	0.1	-3.2	8.6	2.5
F97SI	Harness	9	2	2	2	100	90	90	0.6	0.6	0.6	2.7	1.0	8.3
F00YA	Harness	19	2	1	1	100	60	60	0.2	0.1	0.5	4.4	2.0	5.0

6.3. Results

6.3.1. Predicting stroke rates

We tested 25 different combinations of minimum gradient before peak (n=5 levels; tape: 10-50; harness: 60-100) and running means (n=5 levels; 0.1-4 sec) to smooth data and evaluated the difference between predicted stroke rate and actual stroke rate. Stroke rate could be accurately predicted from finding peaks in the dynamic acceleration of all the axes tested (X, Z and X + Z axes), where no one axis was better at predicting stroke rate than another (Table 6.2). The accuracy of predictions depended predominantly on which gradient and running mean were used. The exact combinations of running mean and gradient required to achieve the lowest error rates for stroke predicted and the number of actual strokes for each dive across both groups was between 0 and 3.1, which represented a percentage difference of between -7.1 and 8.6% (Table 6.2). This demonstrates that stroke rate predictions were both over- and under-estimated depending on the animal and the running mean and gradient used.

The output for the tape group revealed that the choice of axis and running mean were not important in predicting stroke rate correctly, but that a gradient of 40 or 50 was needed (Appendix B). Z-tests revealed that seven of the 25 running mean and gradient combinations were not significantly different from 0. When looking at the output for the harness group, there was no consistency in results for the choice of axis, running mean or gradient. Generally, a higher gradient (>70) and a higher running mean (>2 seconds) resulted in lower errors (Appendix C). Z-tests revealed that eight of the 25 running mean and gradient combinations were not significantly different from 0.

Figure 3 displays the distribution of errors for some selected combinations of gradients and running means. Distributions centred on 0 with small variances represent the best combinations, which differed for each attachment group. The best combination was chosen from the distribution that was not significantly different from 0 (observed = estimated) as determined by z-tests. For otariids with the harness, the best combination was a running mean of 1 second and a gradient of 60 using the x-axis only, as the errors are distributed around 0 with the least variance (Fig. 3C) and the differences were not significantly different to 0 (z = -0.1, p < 0.001; Appendix 1A). For otariids with the accelerometer taped on the best combination was a running mean of 3 seconds and a gradient of 50 using the x-axis only as the errors are distributed around 0 with the least variance (Fig. 3F) and the differences were not significantly different to 0 (z = 0.5, p < 0.001; Appendix 1B).



Figure 6.2 Distributions of differences between predicted stroke rate and actual stroke rate over different running means and gradients. A-C) otariids with the accelerometer taped on (N = 49 trials); D-F) otariids wearing a harness with the accelerometer (N = 71 trials). A and D – X axis; B and E – Z axis; C and F – X + Z axes.

6.3.2. Energy expenditure from stroke rates

Figures 4 and 5 demonstrate the expected over- or under-estimation in energy expenditure from calculating stroke frequency using different gradients and a running mean of three seconds. In Figures 4D and 5D the error is centred around 0, meaning that although on some dives the total number of strokes may be over- or under- estimated, over a long foraging trip these errors will be balanced across the overall energy estimation. However, if a different gradient was used, then the total number of strokes for a dive, and hence, the energy expenditure for that dive was generally underestimated, up to 225 J/kg for the harness group and up to 400 J/kg for the tape group. Most errors fell between ± 100 J/kg excess energy expenditure on each dive on a typical foraging trip of an otariid of 2500-4000 dives (Jeanniard-

du-Dot *et al.* 2016c) could result in a $\pm 250,000-400,000$ J/kg under- or over-estimation of energy expenditure for that trip.



Excess energy expenditure = ± 100 J/kg $\times 3000$ dives = $\pm 300,000$ J/kg $\sim \pm 300$ kJ/kg

Figure 6.3 Potential energy loss or gain from over- or under- estimation of total stroke rate from different gradients for the tape (A-D) and harness (E-H) group. Data were estimated using data smoothed with a running mean of 3 seconds and multiplying the difference in number of strokes observed and estimated for a given dive by the cost of a stroke (7.9J/kg – see methods) for four different gradients for the tape group: A – 20; B – 30; C – 40; D – 50; and for the harness group: E – 60; F – 70; G – 80; H – 90.

6.4. Discussion

This study demonstrates that the parameters chosen to tune the stroke rate calculation from accelerometers can affect the overall estimate. Here we tested different combinations of running means to smooth the data and gradients that determine a peak in the smoothed acceleration that corresponds to a stoke. Accelerometry data consists only of peaks and troughs (Fig. 1), therefore it is important to

choose a gradient that corresponds only to a stroke and not to other movement of the body. Choosing the correct combination of running mean and gradient to predict stroke rate is important because total number of strokes could be under- or over- predicted by $\sim 20\%$. However, if the goal of the research is to predict energy expenditure from strokes then this error will have little effect on the overall estimate. On a typical foraging trip of an otariid performing between 2500-4000 dives the over- or under-estimated energy expenditure would typically be between 200-500kJ/kg. For a 35kg animal this is a gain or loss of 7000-17500kJ over an entire foraging trip. If on a typical foraging trip 140MJ are expended (Jeanniard-du-Dot *et al.* 2016c) the expected gain or loss in energy expenditure is less than 1%. Therefore, while accuracy may be lost from estimating the total number of strokes on a foraging trip, if the goal is to use stroke rate to estimate energy expenditure a relatively wide range of parameters are available to use which will result in low errors.

These results show that for accurate prediction of stroke rate for otariids the gradient used to detect a peak in the accelerometery is the most important parameter. Accelerometers measure the movement of an animal in three directions - surge, heave and sway - and the amplitude of these measurements are dependent on the activity of the animal. The stroke pattern of otariids causes a surge of acceleration forward (x-axis) and upward (z-axis), which results in steep peaks in these two axes (Jeanniard-du-Dot et al. 2016c). Here we have shown that these steep peaks can be identified using a minimum gradient (Fig. 1), which were confirmed to match the strokes of the individual with video analysis. For otariids that have the accelerometer attached with tape a relatively shallow gradient can be used to detect strokes (40-50; Figure 1). For otariids with the accelerometer placed in a harness a steeper gradient was required to account for the noise in the data (90-100; Appendix C). A larger gradient was required for the otariids wearing harnesses because of the additional data generated from the higher sampling rate of the accelerometer (32 Hz vs 25 Hz) and movement from the harness. The running mean chosen did not have a large influence on the overall prediction of stroke rate. Dynamic body acceleration (DBA) is derived from applying a running mean over the axes of acceleration to calculate static acceleration (gravity) and removing this from the raw acceleration (Shepard et al. 2008b). The value used to calculate the running mean changes the value of the DBA, and thus affects the ability of DBA to predict energy expenditure and to calculate an accurate estimate of stroke rate (Shepard et al. 2008a).

Accelerometer attachment (tape or harness), running mean and the axis (or combination of axes) used each had some effect on the results, but overall were not important in accurately predicting stroke rate. For animals wearing a harness, as the accelerometer was recording at a higher rate, a steeper gradient was required as more peaks and troughs were evident in this data. However, sharp jolts by the animal (such as stopping suddenly) did cause a spike in the accelerometry data that was interpreted as a stroke. This did not appear to occur with the animals that had the accelerometer attached with tape. Investigations of wild pinnipeds generally involve the device being glued to the animal, which is analogous to the tape method, suggesting wild studies will not be adversely affected. However, future studies investigating stroke rate in captivity can still use harnesses to attach devices, provided the noise in the data is accounted for by changing the gradient. This is important for animals such as sea lions where attachment of devices with tape in captivity is difficult because the hair is too short (M. Ladds pers. comm.) or when multiple devices must be placed on the animal to measure activity in the open water (Rosen *et al.* 2016).

While the miniaturisation of data-loggers is making it easier to collect data from free-living animals, data storage can still be an issue, particularly if the goal is to monitor the animal over a long period. We found little variation between the ability of a single axis compared to a combination of axis to predict stroke rate. This suggest that if memory or power of a logger that is to be deployed for long durations is limited, it is still possible to obtain good stroke rate measures using only a single axis – x (Halsey *et al.* 2009a) i.e. measuring only this axis allows for robust prediction of stroke rate in otariids. Sample rates of the accelerometers may also affect the ability to predict stroke rate from accelerometers, with lower rates of sampling being more variable, and predictions more robust at rates greater than 0.2Hz (Halsey *et al.* 2009a). As we were measuring well above this frequency (25 and 32Hz) sampling frequency was not a confounding factor when estimating stroke rate in our study. Potentially this means that in wild studies a much lower sampling frequency could be used, saving battery and memory of devices, allowing them to be deployed for much longer durations (Halsey *et al.* 2009a).

6.4.1. Conclusion

Stroke rate may be a useful proxy for estimating energy expenditure (Jeanniard-du-Dot *et al.* 2016c), but as with any proxy, it is important that steps are taken to validate its utility. Here we attached accelerometers, recording at different rates, to fur seals and sea lions swimming under controlled conditions. We found that recording rate of the accelerometer required that a steeper gradient was used to account for the additional data (and thus peaks) recorded from the accelerometer. Further, the running mean selected had little influence on the accuracy of the estimated number of strokes. Changing the gradient used to identify strokes can impact on the overall estimation of energy expenditure, but over an entire foraging trip the error would be less than 1%. Therefore, when using accelerometers to estimate stroke rate for otariids any running mean of between 1 and 4 seconds is appropriate, measured on a single axis (x or z). However, the gradient used must be selected according to the sampling rate of the accelerometer, where a higher gradient is required for a higher sampling rate. This study shows that accelerometers are a simple yet valid tool for estimating the stroke rates of swimming otariids provided care is taken in selecting the appropriate gradient for identifying peaks in the accelerometry.

Chapter 7

Proxies of energy expenditure for marine mammals: an experimental study of "the time trap"

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Abstract

Direct measures of energy expenditure are difficult to obtain in marine mammals, and accelerometry may be a useful proxy, but its utility with marine mammals has recently been called into question. Part of the discussion has related to the way the proxy is calculated. To test this, we measured oxygen consumption of captive fur seals and sea lions wearing accelerometers during submerged swimming to calculate total and a rate of energy expenditure. We then compared these values with three potential proxies of energy expenditure derived from accelerometry data: flipper strokes and two measures of dynamic body acceleration (DBA), overall (ODBA) or vectorial (VeDBA). We tested a range of running means and thresholds required to derive DBA in order to optimise its predictive power and to assess if there is a universal calculation applicable to a range of species differing in size. Total number of strokes, DBA area under the curve (AUC), and submergence time all reliably predicted total oxygen consumption $(sVO_2 \text{ ml kg}^1)$. DBA AUC explained more of the variation than total number of strokes or dive duration, but both DBA AUC and total number of strokes were highly correlated with submergence time. Neither stroke rate nor mean DBA could predict the rate of oxygen consumption (SVO_2 ml min⁻¹ kg⁻¹). Combinations of running mean and threshold for optimal predictions of energy expenditure differed among species and individuals. It appears that the apparently strong relationship of DBA AUC and total number of strokes with total oxygen consumption was a result of incorporating time in both sides of the equation, effectively introducing a constant (time) that is correlated with itself. Removing time from the equation by looking at a rate of energy expenditure, the relationship to both proxies disappears. Further, there appears to be no universal calculation for deriving DBA, further limiting its usefulness as a proxy for estimating energy expenditure. As such, it does not appear to be a useful proxy for estimating energy expenditure in fur seals and sea lions.

7.1. Introduction

Two primary components of the energy expended to acquire prey for marine mammals are the cost of travelling to the foraging destination, and the energy expended from diving, hunting and capturing prey (Rosen and Trites 2002). As air-breathing vertebrates, marine mammals face unique challenges as their prey is patchily distributed throughout the ocean, often in deep water (Harcourt *et al.* 2001). This requires swimming long distances and diving to great depth in ocean waters which entails significant energy expenditure (Costa and Gales 2003, Williams *et al.* 2004b). Measuring this energy expenditure is most accurately done with respirometry systems that measure oxygen consumption, but this method is essentially confined to the laboratory (Weise and Costa 2007, Gerlinsky *et al.* 2013, Ladds *et al.* 2016b). Therefore, to measure the energy expenditure of wild marine mammals an alternative method is sought.

Approaches to make these estimates include measuring heart rate (reviewed in Green 2011), injection of doubly labelled water (DLW) (reviewed in Butler *et al.* 2004), and accelerometry (Fahlman *et al.* 2008b, Jeanniard-du-Dot *et al.* 2016b) from which other proxies of energy consumption can be derived. Monitoring heart rate and using DLW can both give good estimates (Boyd *et al.* 1995b), but can be extremely invasive, may require surgery, are expensive, involve difficult to obtain radionuclides and have been shown to on average overestimate metabolic rate (Butler *et al.* 2004, Dalton *et al.* 2014b). DLW also only provides a single estimate over an extended period, unable to provide fine resolution variation in expenditure with activity. Accelerometry offers an affordable, less invasive and potentially more reliable alternative (Gleiss *et al.* 2011b). It can be used to predict stroke rate (Williams *et al.* 2004b) or a derivative of dynamic body acceleration (DBA), either vectorial (VeDBA) or overall (ODBA) (Qasem *et al.* 2011a), while stroke rate can be calculated from body acceleration measured on three axes (Halsey *et al.* 2011a), while stroke rate can be calculated from the peaks in the dynamic acceleration of the x-axis (Tanaka *et al.* 2001, van Dam *et al.* 2002).

While these methods have demonstrated strong predictive relationships to oxygen consumption in terrestrial animals (Halsey *et al.* 2009b) and birds (Halsey *et al.* 2007, Gómez Laich *et al.* 2011, Sato *et al.* 2011), the results in marine mammals have so far been mixed. Number of strokes was shown to be useful in predicting energy consumption in Weddell seals (*Leptonychotes weddellii*) (Williams *et al.* 2004b), northern fur seals (*Callorhinus ursinus*) and Antarctic fur seals (*Arctocephalus gazella*) (Jeanniard-du-Dot *et al.* 2016c). By contrast, in a sample of Steller sea lions (*Eumetopias jubatus*) swimming to feeding tubes at depth, activity (measured by ODBA) correlated well with oxygen consumption (measured by respirometry) (Fahlman *et al.* 2008b); albeit, this was with a small effect size (see Halsey *et al.* 2011a rebuttal). However, when data were analysed within different dive types using the same animals there was no significant relationship between ODBA and active metabolic rate (Volpov *et al.* 2015b, Volpov *et al.* 2016). A poor relationship between ODBA and energy expenditure (measured via DLW) was found for northern fur seals, which was attributed to trying to incorporate all activity into one measure (Skinner *et al.* 2014). When activity was identified (diving, transiting or resting) a strong relationship was found between VeDBA and energy expenditure (geneniard-du-Dot *et al.* 2016b).

While there does appear to be great promise in the use of accelerometer for measuring energy expenditure (either via stroke rate or a measure of DBA) a recent commentary puts some of these results under scrutiny, and highlights that some of these relationships may have resulted from the "time-trap" (Halsey 2017a). This problem derives from using the sum of the energy expenditure and the sum of the accelerometer output (either number of strokes or DBA), which has the inherent property of including time in both the dependent and independent variable. Regressing these two values with one-another is likely to result in a strong relationship as time is correlated with itself (Halsey 2017a). Therefore, it is the goal of this study to experimentally test the effects of time on the relationship of energy expenditure (as measured via respirometry) both with stroke rate and DBA in a controlled laboratory environment.

7.2. Materials and Methods

7.2.1. Animals

We conducted experiments with three New Zealand fur seals (Arctocephalus forsteri), two Australian fur seals (Arctocephalus pusillus) and four Australian sea lions (Neophoca cinerea) and four Steller sea lions (see Table 7.1 for details of the animals) at four research facilities: Dolphin Marine Magic (RF1: Coffs Harbour, NSW, Australia); Underwater World (RF2: Mooloolaba, QLD, Australia), Taronga Zoo (RF3: Sydney, NSW, Australia) and Open Water Research Station (RF4: Port Moody, BC, Canada). Experiments were conducted between October and December 2014 at RF1-2 and between November and December 2015 at RF4. All animals were on permanent display or were housed for research purposes, were non-reproductive during the study period and were cared for under the husbandry guidelines of the individual facility. All the Australian sea lions were born as a part of a captive breeding program ongoing in Australian aquaria, while all the fur seals came into captivity as juveniles having been found in poor health or injured and were considered unsuitable for release back into the wild after prolonged rehabilitation. All Steller sea lions were collected from breeding rookeries as pups and raised at the Vancouver Aquarium (British Columbia, Canada). All animals were in good health and condition as assessed by the in-house veterinary surgeon at the time of the experiments. Experiments conducted in Australia were approved by Macquarie University ethics committee (ARA-2012_064) and Taronga ethics committee (4c/10/13). All animal handling and experimental procedures in Canada were conducted in accordance with regulations of the Canadian Council on Animal Care (University of British Columbia animal use permit #A11-0397), Department of Fisheries and Oceans Canada (MML 2007-001) and the Vancouver Aquarium.

7.2.2. Trial protocol and metabolic measurements

During all experiments otariids were equipped with a 3-axis accelerometer (RF1-3: CEFAS G6a+, \pm 8g, 40 × 28 × 16.3 mm and mass 18 g in air and 4.3 g in seawater, CEFAS technology Ltd, Lowestoft, UK; RF4: Daily Diary, 95 × 45 × 26 mm, 90 g, Wildlife Computers; Table 7.1). All sea lions (except ASM2)

wore a tight-fitting harness containing the accelerometer while all fur seals (and ASM2) had the accelerometer attached with tape.

Two types of metabolic rate were measured – active metabolic rate (AMR) and standard metabolic rate (MRs). To ensure that the metabolic rates collected during dives were independent, and to measure the effect of activity, we collected oxygen consumption data from the otariids using open-flow respirometry, before and after subsurface swimming. As our experimental conditions prevented us from measuring resting metabolic rate (RMR) we instead measured surface metabolic rate (MRs) as our baseline estimate of metabolic through satisfying as many conditions of Kleiber (1975) as possible (Hurley and Costa 2001). To estimate MRs prior to swimming, otariids would float near motionless under the floating respirometry hood (RF1, RF2 and RF3 - 80 L; RF4 - 100 L) until a consistent baseline rate of oxygen consumption was collected for a minimum of 3 min. Prior to trials otariids had not been fed for a minimum of 14 hours (post-absorptive), were resting in husbandry pools, were adult, not pregnant and remained within their assumed thermo-neutral zone during the trials (as determined by water temperature). Since the thermoneutral zone for the Australian otariid species has not been determined, they were assumed based on the Californian sea lion whose habitat has similar water temperatures (Liao 1990, Liwanag et al. 2009). Water temperature for this study ranged from 18 to 26°C (mean 22.3±3.1) at RF1-3 and 9 to 11°C at RF4 (mean 9.3±0.4). A small amount of food reward was used while otariids were in the floating respirometry hood but based on previous experiments this was not expected to influence the metabolic rates of the otariids (Rosen and Trites 1997, Rosen et al. 2015).

To obtain measures of AMR, otariids would swim submerged for a pre-determined time before returning to the hood where they remained until their instantaneous rates of oxygen consumption returned to within 5% of levels measured prior to swimming (MRs), ensuring that all dives were independent as otariids recovered fully between each dive. To estimate AMR, sea lions at RF4 dived to 10m where they received small pieces ($\sim 20 \text{ g}$) of herring at a 5 or 10 second rate while swimming between two submerged feeding stations between 1 and 3 m below the water's surface (Rosen *et al.* 2016), while otariids at RF1-3 were trained to swim laps of a pool between two stationary targets (Ladds *et al.* 2016a). All animals were familiar with the experimental equipment and performed all trials voluntarily under trainer control. Submergence durations were timed *in situ* at all facilities and confirmed with video footage. The distance covered and submergence time of trials for otariids differed due to differences in experimental set-up, training differences, and motivation of the seal on the day of the trial. Some trials were incomplete due to the seal surfacing outside of the hood and these were excluded from the analysis.

The respirometry hood was connected to an open-flow respirometry system (Sable Systems International, Inc., Henderson, NV, USA). Air was pulled from the hood with a Sable Systems Mass Flow pump at an adjustable flow rate ranging from 300-475 l min⁻¹, depending on the size of the animal. A dried continuous sub-sample of expired air was drawn into the oxygen analyser from the pump and average concentrations were recorded at 5 samples a second at RF1-3 and 2 samples a second at RF4. Rates of oxygen consumption ($\dot{V}O_2$) were calculated using equation 4b from Withers (1977) assuming a
respiratory quotient of 0.77 (Feldkamp 1987). To determine the mass-specific total energy expenditure $(sVO_2 \text{ ml kg}^{-1})$ used during a trial the total amount of oxygen consumed during post-dive that was greater than pre-dive consumption rates was integrated and divided by mass (kg) (Williams *et al.* 2004b). To obtain a mass-specific rate of energy expenditure $(s\dot{V}O_2 \text{ ml min}^{-1} \text{ kg}^{-1}) sVO_2$ was divided by the submerged duration and the recovery period (Fahlman *et al.* 2008b). Only dives that had a recovery period of longer than 120 seconds were kept for analysis.

Mass (± 2 kg) was recorded once per week of trials for otariids housed at RF1, RF2 and RF3 as a part of their normal routine and at RF4 sea lion mass (± 0.5 kg) was measured daily.

7.2.3. Accelerometer measurements

Accelerometers (described above) recorded time, depth, and acceleration on 3 axes: anterior-posterior (surge, x-axis), lateral (sway, y-axis) and dorso-ventral (heave, z-axis), from which ODBA, VeDBA and stroke frequency of dives, were extracted.

7.2.3.1. ODBA and VeDBA

Both the estimate for overall dynamic body acceleration (ODBA, g) and vectorial dynamic body acceleration (VeDBA, g) changes with the running mean selected (Shepard *et al.* 2008a). We chose to test a range of running means and evaluated how this affected the overall relationship with oxygen consumption. Additionally, as sVO_2 (ml kg⁻¹) and $s\dot{V}O_2$ (ml min⁻¹ kg⁻¹) only accounts for the energy that is expended above resting, it is theoretically possible to remove the passive component of movement within a swim/dive cycle (where we assume the seal is using their resting metabolism) by removing a threshold (baseline) value. Therefore, we also tested the effects of incorporating thresholds of 0, 0.1, 0.2, 0.3 and 0.4g on predictive capacity (an example of how these work is shown in Figure 1.

To calculate ODBA and VeDBA static acceleration must first be derived. We calculated static acceleration for each axis using a range of running means: 0.4, 1, 2 and 3 seconds. An estimate of dynamic acceleration was then obtained by subtracting the static acceleration from the raw values. Then, to calculate ODBA the absolute values of each of the dynamic estimates were summed (Eq. 7.1) and to calculate VeDBA the square root of the summed dynamic estimates is calculated (Eq. 7.2).

$$ODBA = |X_{dyn}| + |Y_{dyn}| + |Z_{dyn}|$$
(7.1)

$$VeDB = \sqrt{X_{dyn}^2 + Y_{dyn}^2 + Z_{dyn}^2}$$
(7.2)

ODBA and VeDBA mean and area under the curve (AUC) were calculated every combination of a running mean of 0.4, 1, 2, 3 or 4 seconds and thresholds of 0, 0.1, 0.2, 0.3 or 0.4.



Time (seconds)

Figure 7.1 ODBA (g) calculated with a running mean of 2 seconds from a 60 second swim with a comparison of the overall mean ODBA estimated using different thresholds.

7.2.3.2. Stroke frequency

The dynamic body acceleration (DBA) of the x-axis determined was also used to calculate stroke frequency, the details of which have been detailed elsewhere (Chapter 5). Briefly, a sub-sample of swims during trials were recorded using an underwater camera (GoPro) so that stroke frequency could be directly calculated. The peaks in the x-axis were identified from data that had been smoothed with a running mean of three seconds. Strokes were identified as peaks that exceeded a threshold, determined through experimentation (Chapter 5).

7.2.4. Statistical analysis

7.2.4.1. Groups of animals

Animals were delineated into three separate groups for analysis based on their development and sex as these were previously shown to affect metabolic rate, whereas species did not (Ladds *et al.* 2016b). The resulting groups were small females and juveniles (combined as they had similar masses and have previously been shown to have similar metabolic rates), adult males and large females (Steller sea lions) (Table 7.1). The large females were separated from males as they were significantly larger and had a different experimental set-up to the other animals.

In total, there were 100 variables created for DBA (ODBA and VeDBA, both mean and AUC) from combinations of running means and thresholds. Pearson's correlation coefficient was used to select the variables that demonstrated the strongest correlations with sVO_2 and $s\dot{V}O_2$ that were then used in the models.

7.2.4.3. The models

We used multiple linear mixed-effects models (LME) with restricted maximum likelihood (REML) estimation to evaluate which source of variation best explained change in sVO_2 (ml kg⁻¹) and $s\dot{V}O_2$ (ml min⁻¹ kg⁻¹) (NLME package in R; Pinheiro *et al.* 2014). Using sVO_2 and $s\dot{V}O_2$ as the response variables, we first ran null models (no random effects) to find a baseline from which we could evaluate the influence of the random effect on the models. We then ran LME's with individual animal as the random effect to account for repeated measures. The predictor variables for the sVO_2 model were: submergence duration, total strokes and VeDBA or ODBA AUC. The predictor variables for the $s\dot{V}O_2$ model were tested with species, sex and attachment method as co-variates to determine their influence on the models. The best combination of variables were tested using the function *dredge* from the package MuMln in R.

Model selection was based on a combination of Akaike Information Criteria (AICc), log likelihoods (logLik) and R². The amount of variance explained by the random effect was assessed through the difference of the marginal (fixed effect only) and conditional (all model variables) R² (rsquared.glmm function). The assumptions of homoscedasticity, normality, homogeneity and independence were investigated by plotting predicted vs fitted residuals, QQ-plots, Cleveland dot-plots and ACF plots (Zuur *et al.* 2009b). Where models did not meet assumptions, we log transformed the predictor and/or the independent variable. All analysis was completed in R (Version 3.1.3; R Core Development Team 2015) and values are reported as mean \pm SD.

7.3. Results

7.3.1. Rates of oxygen consumption

Animals completed between 7 and 35 trials and these ranged in duration from 26 to 221 sec. A trial was defined as one submerged swim with a complete recovery. Larger animals on average remained submerged for longer than smaller animals (Table 7.1). sVO_2 ranged from 5.44 to 115.00 (ml kg⁻¹) and $s\dot{V}O_2$ ranged from 6.49 to 41.67 (ml min⁻¹ kg⁻¹).

7.3.2. Dynamic body acceleration (DBA)

We tested 25 combinations of different thresholds (0-0.4) and running means (0.4-4) to calculate the mean and area under the curve of DBA (ODBA and VeDBA) which were correlated against $s\dot{V}O_2$ (ml min⁻¹ kg⁻¹) and sVO_2 (ml kg⁻¹) respectively (Fig. 7.2). We found that different combinations of thresholds

and running means for different groups greatly influenced the overall correlation with both $s\dot{V}O_2$ (ml min⁻¹ kg⁻¹) and sVO_2 (ml kg⁻¹), but there was very little difference when using ODBA or VeDBA. The most effective running mean for predicting $s\dot{V}O_2$ (ml min⁻¹ kg⁻¹) differed across the three groups, where a 0.4 second running mean was best for large females (Fig. 7.2A) and small females and subadults (Fig. 7.2C), while a 2 or 3 second running mean was best for males. Using a threshold did not provide any clear improvement of the correlation of $s\dot{V}O_2$ with ODBA and VeDBA. However, for small females and subadults, a shorter running mean (0.4 sec) improved the correlation of DBA with sVO_2 (ml kg⁻¹), except that using a running mean of 4 second reduced the correlation (Fig. 7.2D-E). Unlike for $s\dot{V}O_2$, for all groups using a threshold consistently improved the relationship of DBA with sVO_2 (ml kg⁻¹), where the large threshold corresponded to a higher correlation.

7.3.3. Predicting energy expenditure

LME's were used to predict the relationship of sVO_2 and $s\dot{V}O_2$ with the best combination of running mean and threshold for both measures (ODBA and VeDBA) of DBA AUC and mean DBA for each animal group. The effect of attachment type and location were tested in each of the models and neither improved the AIC or the variance explained. Therefore, they were not considered further. For all combinations of LME's adding individual as a random effect improved the variance explained. For all groups $log(sVO_2)$ (ml kg⁻¹) could be accurately predicted from submergence time (R² fixed = 0.67-82; Fig. 7.3A-C) with individual accounting for between 8 and 21% additional variation (Table 7.2). Both the total number of strokes and VeDBA AUC could also predict $log (sVO_2)$ (Fig. 7.3B and 7.3C), but both variables were highly co-linearly related to swim duration (Fig. 2G-H). VeDBA explained more of the variance in $log (sVO_2)$ (ml kg⁻¹) than submergence time or total strokes (Table 7.2).

The strongest predictor of $log (s\dot{V}O_2)$ (ml min⁻¹ kg⁻¹) was submergence time for all groups, due to a negative relationship where $log(s\dot{V}O_2)$ (ml min⁻¹ kg⁻¹) decreased with increased submergence time (Fig 7.3D; Table 7.2). There was no relationship between stroke rate (Hz) and $s\dot{V}O_2$ (Fig. 7.3E) or mean VeDBA and $s\dot{V}O_2$ (Fig. 7.3F) for all groups.



Figure 7.2 Pearson's correlation coefficients for relationships between combinations of different running means and thresholds of ODBA and VeDBA with sVO_2 (ml kg⁻¹; A-C) and $s\dot{V}O_2$ (ml min⁻¹ kg⁻¹; D-F). A and D) large females diving (N = 4 animals; n = 130 trials); B and E) male fur seals and sea lions swimming transitionally (N = 5 animals; n = 86); C and F) female and juvenile fur seals and sea lions swimming transitionally (N = 4 animals; n = 47 trials).

Table 7.1 Seal characteristics, accelerometer details and summary metabolic rates from all trials. Mean (\pm SD) and number of trials for sVO_2 (ml kg⁻¹) and $s\dot{V}O_2$ (ml min⁻¹ kg⁻¹) measured after activity, with time spent submerged (mins), species, ID, mass (kg), age (years) and marine facility where housed, type of accelerometer used, recording rate and method of attachment for five fur seals and eight sea lions. Marine facility: RF1 – Dolphin Marine Magic; RF2 – Underwater World; RF3 – Taronga Zoo; RF4 – Open Water Research Station. Species: AFS – Australian fur seal; ASL – Australian sea lion; NZFS – New Zealand fur seal; SSL – Steller sea lion

Species	ID	Mass	Age	Marine facility	Device	Recording rate	Attachment method	Submergence time	N	sVO ₂	sŸO ₂
Small females and juveniles											
AFS	AFF1	69-78	17	RF2	G6a+	25Hz	Таре	1.46 (0.20)	12	45.28 (8.47)	21.01 (2.94)
ASL	ASF4	66	17	RF1	G6a+	25Hz	Harness	1.43 (0.58)	7	45.72 (27.89)	22.35 (7.04)
ASL	ASF1*	47	5	RF1	G6a+	25Hz	Harness	1.39 (0.12)	8	56.22 (6.93)	29.43 (3.06)
NZFS	NFM1*	54-55	8	RF3	G6a+	25Hz	Таре	1.03 (0.19)	7	36.13 (9.69)	34.52 (5.74)
Large ma	ales										
AFS	AFM1	179-182	14	RF2	G6a+	25Hz	Таре	1.45 (0.36)	24	35.58 (11.26)	17.68 (5.02)
ASL	ASM1	153-160	12	RF3	G6a+	25Hz	Таре	1.35 (0.32)	17	28.65 (8.43)	15.37 (3.03)
ASL	ASM2	110-125	9	RF1	G6a+	25Hz	Harness	2.03 (0.31)	7	68.02 (12.67)	16.87 (3.39)
NZFS	NFM2	149-161	11	RF2	G6a+	25Hz	Таре	2.17 (0.44)	23	50.77 (17.97)	10.64 (1.59)
NZFS	NFM3	154	13	RF3	G6a+	25Hz	Таре	0.95 (0.10)	15	16.47 (2.58)	18.42 (2.04)
Large fer	nales										
SSL	F00BO	155-160	15	RF4	Daily Diary	32Hz	Harness	2.31 (0.75)	33	53.84 (25.55)	10.52 (3.87)
SSL	F97HA	172-175	18	RF4	Daily Diary	32Hz	Harness	2.19 (0.81)	33	54.58 (27.30)	11.94 (3.46)
SSL	F97SI	230-233	18	RF4	Daily Diary	32Hz	Harness	2.38 (0.67)	29	53.53 (22.37)	9.49 (1.63)
SSL	F00YA	214-218	15	RF4	Daily Diary	32Hz	Harness	2.35 (0.85)	35	56.78 (30.23)	10.42 (2.59)

*Indicates seals identified as subadults during trials.

Table 7.2 Results of linear mixed effects models. Relationships presented are between total energy expenditure (ml kg⁻¹) and rate of energy expenditure (ml kg⁻¹ min⁻¹) with submergence time (mins), dynamic body acceleration (g) and strokes.

Response	Predictor	Group	Equation	R ² fixed	R ² all	LogLik	AIC
Log of total		Males	$-2.29 + \log(x) 1.30$	0.82	0.94	42.11	-76.21
energy expenditure	Submergence time	Females/ subadults	$-2.27 + \log(x)1.33$	0.72	0.93	11.62	-15.23
		Large females	$1.03 + \log(x)0.43$	0.67	0.75	101.82	-195.64
	Strokes RM:4 sec; G:30	Males	$-3.12 + \log(x)1.33$	0.58	0.82	2.13	3.74
	Strokes RM:4 sec; G:30	Females/ subadults	$3.05 + \log(x)0.03$	0.40	0.49	-0.51	9.02
	Stroke RM:1 sec; G:100	Large females	$1.77 + \log(x)0.43$	0.63	0.71	94.00	-180.00
	VeDBA AUC RM:1 sec; T:0.3	Males	$-2.27 + \log(x)1.55$	0.89	0.89	23.69	-39.39
	VeDBA AUC RM:0.4 sec; T:0.2	Females/ subadults	$-0.44 + \log(x)1.33$	0.91	0.93	22.75	-37.50
	ODBA AUC RM:3 sec; T:0.4	Large females	$1.42 + \log(x)0.43$	0.70	0.75	104.41	-200.81
Log of rate		Males	1.66 - 0.009(x)	0.71	0.87	38.91	-69.83
of energy expenditure	Submergence time	Females/ subadults	1.02 – 0.007(x)	0.53	0.66	13.65	-19.30
		Large females	$3.47 - \log(x)0.57$	0.59	0.88	99.40	-190.80
	RM:4 sec; G:30	Males	Not significant	0.03	0.66	1.33	5.35
	RM:4 sec; G:30	Females/ subadults	Not significant	0.03	0.54	8.80	-9.60
	RM:1 sec; G:100	Large females	Not significant	0.04	0.27	-3.05	14.01
	Mean VeDBA RM:3 sec; T:0.4	Males	Not significant	0	0.64	-2.52	13.03
	Mean VeDBA RM:0.4 sec; T:0.2	Females/ subadults	Not significant	0	0.35	5.75	-3.35
	Mean ODBA RM:3 sec; T:0.4	Large females	Not significant	0.02	0.24	-5.01	-18.02



Figure 7.3 Relationship between recovery oxygen consumption (sVO_2 ml kg⁻¹; top panel) and swim duration (A), number of strokes (B) and VeDBA AUC (C) and relationship between diving metabolic rate ($s\dot{V}O_2$ ml min⁻¹ kg⁻¹; bottom panel) and swim duration (D), stroke rate (E) and average VeDBA (F). The relationship between swim duration and of total number of strokes (G) and VeDBA AUC (H) are displayed for comparative purposes. Open circles are small females and subadults (N = 4 animals; n = 47 trials), closed grey circles are males (N = 5 animals; n = 86) and closed black circles are large females (N = 4 animals; n = 130 trials). For comparisons with other papers the average VeDBA used in F has a running mean of 2 seconds and no threshold (Shepard et al. 2008; Fahlman et al. 2008). *Represents an outlier that was removed when fitting the regression.

7.4. Discussion

The relationship between energy expenditure and proxies, such as stroke rate or measures of DBA (e.g., VeDBA or ODBA) for diving mammals has recently been brought under question (Halsey 2017b). Here we provide further evidence in support of Halsey's contention that the strong relationships observed between total (summed) energy expenditure and total number of strokes or VeDBA AUC (summed) is time correlated with time, using a range of otariids of different ages, sizes, sexes and species. There is a strong positive relationship between total energy expenditure (**sVO**₂ ml kg⁻¹) and time, total number of strokes and VeDBA AUC. However, the apparent relationships observed are actually the result of time correlated with itself, as total number of strokes and VeDBA AUC are highly collinearly related to submergence time. Using VeDBA appeared to help improve this relationship, by accounting for some of the additional variance associated with body movement that occurs during a swim. Further, the ability of DBA (ODBA or VeDBA) and stroke rate to predict energy expenditure changes depending on the running mean and threshold that is used to calculate VeDBA and how the number of strokes are counted (Chapter 6) for different groups of animals. This indicates that it is unlikely for there to be a universal equation to estimate the appropriate DBA for a given individual, limiting further the applicability of this method for estimating energy expenditure in the wild.

7.4.1. Groups of animals

Differences in sex and development stage of the animals and experimental set-ups meant that three distinct groups were created for analysing the results: small females and juveniles, large males and large females (Steller sea lions). When examining sVO_2 , large males and females were indistinguishable, while small females and juveniles had higher total energy expenditures (Figures 7.3A-C), however, when examining the $s\dot{V}O_2$ (ml min⁻¹ kg⁻¹), the three groups do differentiate (Figures 7.3D-F). These differences likely arose due to a combination of different age classes, body sizes and different experimental set-ups for the groups. Large females made dives down to 10m where they swam between feeding tubes where they received fish at a 5-10 second rate while males, small females and juveniles swam laps of pools without stopping. Small females and juveniles generally stroked consistently throughout their swim, while males generally used one or two strokes after turning followed by a long glide. During trials, large

females were often observed stationary at feeding tubes and would use one to two strokes to transition between each one. At these times, and during gliding periods for the males, it is expected that the animals would be using their resting metabolic rate (Williams *et al.* 2004b) or lowering their metabolic rate below these levels (Fahlman *et al.* 2008b). Considering that the males and large females had similar dive durations, stroke rates and were similar masses, it is not surprising that they expended the same amount of total energy over similar dive durations. Small females and juveniles have elevated metabolic rates in comparison to adult males of the same species, which explains their higher metabolic rate in this study (Ladds *et al.* 2016b).

7.4.2. Testing parameters for establishing DBA

When using accelerometers to establish proxies of energy expenditure the decisions made during the derivation of ODBA or VeDBA affect its ability to predict (Halsey *et al.* 2009a). This is often an underappreciated source of methodological variation in these techniques. In this study we used two types of DBA: either summing the absolute (ODBA) or taking the square root of the sum (VeDBA) of the dynamic acceleration (Qasem *et al.* 2012). Dynamic acceleration is derived from applying a running mean over the axes of acceleration to calculate static acceleration (gravity) and removing this from the raw acceleration (Shepard *et al.* 2008b). The value used to calculate the running mean changes the value of the DBA, and thus affects the ability of DBA to predict energy expenditure and to calculate an accurate estimate of stroke rate (Shepard *et al.* 2008a).

Different combinations of the parameters changed the values of both the DBA and the predicted stroke rate (Chapter 6). The large effect of these combinations arose from a number of factors attributable to either the animal or the device. Considering that the males and the large females were roughly the same size during trials, differences were most likely due to sampling frequency and placement of the accelerometer. The accelerometer fitted to large females recorded at 32Hz and was secured in a harness while the accelerometer fitted to males recorded at 25Hz and was taped directly to the fur. There was more movement, and thus more signal changes, in the accelerometer on the harness. In the wild, accelerometers are generally attached to fur with glue, thereby reducing the amount of noise in the accelerometry signal. In this experiment, taping the accelerometer to seals more closely resemble this method. Therefore, when extrapolating these results, the combinations predicted for males will return the most accurate estimate for VeDBA and number of strokes.

7.4.3. Estimating energy from accelerometers: The time trap

Evidence for the relationship between total strokes and total energy expenditure has been shown for a number of species: Weddell seals (Williams *et al.* 2004b), northern elephant seals (*Mirounga angustirostris*) (Maresh *et al.* 2015), and Antarctic fur seals and northern fur seals (Jeanniard-du-Dot *et al.* 2016c). Summed VeDBA and total energy expenditure were highly correlated in diving cormorants (Halsey *et al.* 2011b, Stothart *et al.* 2016) and for Northern fur seals and Antarctic fur seals when the energy expenditure was estimated from different activities (foraging, transiting, surface movement and resting) (Jeanniard-du-Dot *et al.* 2016b). Our study also shows a strong relationship of total energy expenditure

 $(sVO_2 \text{ (ml kg^-1)})$ with summed VeDBA (VeDBA AUC) and total strokes (Fig. 7.2B-C). Similar to Antarctic and Northern fur seals, we found that VeDBA AUC was a better predictor of total energy expenditure than total strokes or dive duration, albeit marginally (Table 7.2; Jeanniard-du-Dot *et al.* 2016c). Dive duration also predicted sVO_2 (ml kg⁻¹) slightly better than total strokes. This contrasts with a study on Weddell seals where the total number of strokes was a better predictor of total energy expenditure than dive duration (Williams *et al.* 2004b). This is likely a result of using gliding during a large proportion of their dive, while our seals were stroking fairly consistently throughout each trial with many changes in body orientation. DBA was able to pick up on these changes and incorporated the cost of the movement into the model. However, while DBA may be able to pick up of some of the randomness in the relationship due to body movement, most of the variance explained is from the incorporation of time into both the independent and dependent variables. This is demonstrated here in the very strong relationships of VeDBA AUC and total strokes with time (Fig. 7.2G-H). This so-called "time-trap" means that counting strokes or measuring VeDBA may be no better than simply using the duration an animal spends diving to estimate the cost of that dive (Maresh *et al.* 2015).

The effect of the time trap is readily apparent when investigating the rate of energy expenditure, that is, by removing time from the equation. When time was removed by expressing the independent ($s\dot{V}O_2$ (ml min⁻¹ kg⁻¹)) and dependent variables (mean VeDBA or stroke rate) as rates, no such relationship was evident (Fig. 7.2D-F). It has been noted that correlations of mean DBA with a rate of energy expenditure in mammal divers may be difficult to establish if oxygen stores were not replenished to the same level between each dive, resulting in inaccurate measures of metabolic rate (Fahlman *et al.* 2008a). We accounted for this by measuring a baseline before each trial and ensuring that metabolic rates returned to within 5% of this value before attempting another trial.

While our study suggests that converting our measures to rates weakens the relationship between energy expenditure and DBA, other studies suggest that the effect of time scale may be more complex. When measuring average partial DBA (PDBA) and $s\dot{V}O_2$ in turtles there was no relationship for single dives but a strong relationship was evident for bouts of diving (Enstipp *et al.* 2011). In cormorants, average daily ODBA and VeDBA correlated with mass-specific daily energy expenditure measured from DLW (Stothart *et al.* 2016), but ODBA did not correlate with $s\dot{V}O_2$ over a single dive cycle (Halsey *et al.* 2011b). By comparison, when the relationship was examined in otariids there was no relationship between mean ODBA and $s\dot{V}O_2$ single dives or during bouts of diving (Volpov *et al.* 2015b, Volpov *et al.* 2016).

Potentially, to find a relationship between stroke rate or mean ODBA and the rate of energy expenditure the swimming intensity could be modified. Diving mammals optimise their stroke patterns and body movements to dive efficiently (Sato *et al.* 2007) and lower the cost of transport (Rosen and Trites 2002, Ladds *et al.* 2016b). This can be observed in the very small amount of variation in the stroke rate and mean VeDBA of the three groups of animals. The otariids from this study were familiar with the experimental set-up, thus they apparently used their most efficient swim pattern, as this is the best way to save energy and reduce the cost of transport (Ladds *et al.* 2016b). To truly test the relationship of the

rate of energy expenditure seals could be trained to experimentally change their stroke output and if possible vary the ODBA output as well. In this study, large females used several strokes to swim to the feeding tubes and would often wait at feeding tubes for fish to arrive. They would then use one or two strokes to transition to the other feeding tube. Large males swam constantly, generally completing laps of the pool with one to two strokes at each turn with a long glide in between. The swimming differences make it possible to see that a relationship between mean VeDBA and $s\dot{V}O_2$ (ml min⁻¹ kg⁻¹) may occur if a larger range of mean VeDBA values could be obtained from increased swimming effort (Fig. 7.3F).

Conclusions and future directions

Measuring the energetic expenditure of free-living marine mammals is fundamental to understanding how they are coping, and predicting how they will cope with environmental changes. Accelerometers showed great promise in being able to measure energy expenditure over long deployments, but the results of this study seem to support a recent commentary that suggested this technique does not measuring the amount of energy expended from an activity, but instead measures the amount of time in that activity. However, measuring the energy expenditure of a free living marine mammal is not as simple as measuring time spent active. The size, sex and age class of the individual must be known to accurately estimate energy expenditure (Ladds *et al.* 2016b). Further, intrinsic and extrinsic factors must be considered. For example, pregnancy (Maresh *et al.* 2015) and temperature (Liwanag *et al.* 2009, Ladds *et al.* 2017a) change the metabolic rates of some pinnipeds. In addition, time spent active, resting and grooming should be considered as each comes with its own energetic cost (Liwanag 2010, Battaile *et al.* 2015, Ladds *et al.* 2017a). Therefore, accelerometers may be useful to derive activity budgets to estimate energy expenditure (Jeanniard-du-Dot *et al.* 2016b, Ladds *et al.* 2016c), though it appears unwise to use them to estimate energy expenditure directly.

Chapter 8

Accelerometers recording at low frequencies can be used to develop timeenergy budgets of wild fur seals from captive surrogates

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Abstract

Accurate time-energy budgets summarise an animal's energy expenditure in a given environment and are potentially a sensitive indicator of how an animal responds to changing resources. Deriving accurate time-energy budgets requires a precise measure of time spent in different activities, and an estimate of the energetic cost of that activity. Diving and movement data were collected from nine wild juvenile Australian fur seals equipped with tri-axial accelerometers. To validate time-energy budgets for the fur seals, energetic and behavioural experiments were conducted with twelve captive surrogates. The time fur seals spent in four behavioural states: foraging, grooming, travelling and resting, was quantified with low and high resolution data from accelerometers using gradient boosting models (GBM). We estimated daily energy expenditure of fur seals (DEE) using a relatively simple energetics model developed from their behaviour and location (land, surface or underwater). Models developed from captive seals were applied to accelerometry data collected from wild juvenile Australian fur seals and their time-energy budgets were reconstructed. Juvenile fur seals expended more energy than adults of similar species, but there was no significant difference in DEE across sex or season (winter or summer). Juvenile fur seals used behavioural compensatory techniques to conserve energy during activities that were expected to have high energetic outputs (such as diving). Developing time-energy budgets from accelerometers is an efficient method of estimating energy expenditure from individuals over time.

Keywords

Accelerometer, fur seal, time-energy budget, daily energy expenditure (DEE)

8.1. Introduction

An animal's fitness can be assessed by its ability to survive and reproduce in a given environment (Orr 2009). Time-energy budgets are a useful measure of one aspect of animal fitness, as they describe the energy spent and energy gained over a specific period in an animals life (Boyd 2002). Energy gains are made by eating and metabolising food, while the key components of energy expenditure are the basal metabolic rate (BMR), digestion, thermoregulation and activity (Costa and Williams 1999). The energy that animals gain in excess of these basic functions is used for growth and reproduction. While the BMR and the cost of digestion are generally static in a constant environment, thermoregulation and activity costs vary depending on the activity and the needs of the animal (Goldstein 1988). Therefore, monitoring the activity patterns of animals over time can determine whether they are in positive energy balance, such that they are able to invest excess energy into growth and reproduction. To make estimates of energy expenditure we need to develop time-energy budgets that quantify both the time animals spend engaged in different activities, and the energetic costs associated with those activities (Travis 1982).

As air-breathing mammals that persist primarily on a diet of aquatic animals, fur seals have challenging constraints when finding energy. Fur seals must perform dives repeatedly when foraging and travel long distances in order to find their food, an energetically intensive strategy (Staniland *et al.* 2007). They are constrained by their need to return to the surface to breathe, and can only dive for as long as their oxygen stores allow (Gerlinsky *et al.* 2014b). The metabolic rate is the rate at which they use those stores, and is a useful measure of the energy process (Ponganis *et al.* 2011). As relatively small marine mammals, fur seals have high thermoregulatory costs when at sea, as water conducts heat 25 times faster than air (Hind and Gurney 1997). While they do use behavioural strategies to mitigate some of these costs, these strategies can still be energetically expensive (Liwanag 2010).

Developing time-energy budgets for wild fur seals helps us to understand how fur seals allocate energy to key processes and are most commonly measured as daily energy expenditure (DEE). DEE is measured by recording the duration of various activities and multiplying these by their associated energetic cost (Goldstein 1988). Calculating the costs associated with different activities from wild populations of fur seals is difficult and often expensive. Instead, estimates of the energetic costs associated with different activities have been made from laboratory experiments using captive surrogates and respirometry (Nagy *et al.* 1999, Liwanag 2010, Ladds *et al.* 2016b). Respirometry is an accurate method of measuring metabolic rate (and thus energy expenditure) but has limited field applications (Ponganis *et al.* 1993, Halsey 2011). Therefore, a proxy of metabolic rate for different activities must be developed using alternative methods, such as devices that can be deployed on wild animals. Technology has provided a range of possible methods that may be used for measuring animal activity and energy expenditure, but it is now the role of biologists to validate the technology (Nathan *et al.* 2012). Animal-borne sensors, such as accelerometers, are a common tool for determining animal activity (Cooke *et al.* 2004, Bograd *et al.* 2010) and have the potential to measure energetic variables as well (Cooke *et al.* 2014, Hussey *et al.* 2015).

Accelerometers have been used to define the behavioural state of a range of animals (Diosdado et al. 2015, Hammond et al. 2016). They can measure specific events, such as prey-capture (Carroll et al. 2014), identify a range of behaviours (Whitney et al. 2010), define movement patterns (Shepard et al. 2008b) and estimate the energy expended from those events (Jeanniard-du-Dot et al. 2016b). Movement costs are the energy expenditure above maintenance and can be estimated from accelerometers when they are positioned close to the centre of the animals mass (Halsey et al. 2009a). Calculation of dynamic movement from the accelerometer can act as a proxy for energy expenditure as it measures the muscle movement of the animal (Gleiss et al. 2011b). Detailed calibrations of the relationship between acceleration profiles and energetic costs are required in order to make reliable estimates (Wright et al. 2014), and these are understandably difficult to make in the wild (Crossin et al. 2014). In addition, the relationship between dynamic acceleration and energy expenditure is dependent on the behaviour as different behaviours have different energetic costs (Gleiss et al. 2011b, Jeanniard-du-Dot et al. 2016b). While accelerometers can record at high resolution (multiple samples per second) which gives a detailed picture of behaviour, battery and memory limitations mean that the period that can be sampled at a high resolution is relatively short compared to the time animals are at sea (Halsey et al. 2009a). As fur seals make long-duration foraging trips over multiple days or weeks, monitoring such trips may require low resolution recording due to the memory constraints of bio-loggers. Therefore, the goal of this study was to evaluate whether we could accurately classify and cost activities derived from captive surrogates using low resolution accelerometry data and apply it to wild fur seals.

8.2. Materials and Methods

8.2.1. Field work

Juvenile Australian fur seals were tagged at two colonies; six at Seal Rocks (Phillip Island, Victoria Australia, 38°52'S - 145°11'E) during austral summer of 2013 and eight at Lady Julia Percy (Victoria, Australia, 38°52'S – 142°00'E) during austral winter of 2014. These two sites represent the largest breeding colonies for Australian fur seals, with each site containing approximately 25% of the species (Kirkwood *et al.* 2010). In Australian fur seals, puberty occurs in females at approximately 3 years old and in males at 4-5 years old with suckling ceasing after 1 year (Arnould and Warneke 2002). We targeted animals between one and three years of age that were independently foraging.

Juveniles were captured using a modified hoop-net and sedated with isofluorane gas (Kirkwood *et al.* 2006). Standard morphometric measurements of length and girth were made to the nearest cm, and mass was recorded using a Pesola[®] scale at \pm 0.5kg. To ensure sampling of juveniles, as opposed to pups or sub-adults, only animals with a mature pelage (not the chocolate pelage of premoult pups) but still less than 1.5m and less than ~40 kg were selected (Arnould and Warneke 2002). Seals were tagged with numbered plastic tags (Super Tags[®], Dalton I.D. Systems Ltd, Henley-on-Thames, UK) placed in the trailing edge of both fore-flippers to aid with identification and recapture. One of two types of location device (Kiwisat100, Sirtrack Ltd, New Zealand or Mk10, Wildlife Computers) a small VHF transmitter

(Sirtrack Ltd, 6cm x 3cm x 2cm), and an accelerometer G6A+ (CEFAS technology Ltd, Lowestoft, UK) were glued directly to the fur on the dorsal midline of each seal using quick-setting epoxy (Araldite 2017, Aeropia Ltd, Crawley, UK or Araldite 268, Huntsman Advanced Materials, Victoria, Australia). Animals were observed until they had fully recovered from anaesthesia and were released at the site of capture. A minimum of 30 days lapsed before seals were recaptured and location transmissions of the seals were downloaded daily to ensure the tags viability. Seals were recaptured using a hoop net and held manually while devices were retrieved by cutting the hair beneath the glued instrument.

TDRs recorded diving parameters (depth, light level, wet/dry) every second, while accelerometers recorded acceleration on three axes at 1Hz, depth at 1Hz and temperature at 0.5Hz for the duration of the deployment, acceleration on three axes was also recorded at a fast rate (20Hz) when diving (depth > 1.5m). Satellite tags were programmed to transmit a pulse signal every 45 seconds when at the surface. Satellite locations and their associated location quality estimate were provided by Collecte Localisation Satellites Argos (Toulouse, France).

8.2.2. Dive segmentation

To determine dive trip parameters data from the MK10 devices were summarised. Any drift in the pressure sensors or error spikes were corrected prior to analyses using Zero-Offset Correction (Wildlife Computers ©) A trip was defined as any period that the tag was wet and the seal performed dive bouts (i.e. any periods in the water with minimal diving, and only shallow dives <10m, were not considered trips). Dives were defined as periods the seal spent underwater below a minimum depth of 5m to account for wave action at the surface. Trip duration, number of trips, average dive duration, mean maximum depth and maximum depth were derived from these parameters.

8.2.3. Behaviour segmentation / Activity budgets

We conducted experiments on captive seals consisting of two Australian fur seals (*Arctocephalus pusillus doriferus*), three New Zealand fur seals (*Arctocephalus forsteri*), one subantarctic fur seal (*Arctocephalus tropicalis*), and six Australian sea lions (*Neophoca cinerea*) (Table 8.1), from three Australian marine facilities: Dolphin Marine Magic, Coffs Harbour (RF1: 30°17'S, 153°8'E); Underwater World, Sunshine Coast (RF2: 25°40'S, 153°7'E); and Taronga Zoo, Sydney (RF3: 33°50'S, 151°14'E). Experiments were conducted from August to November 2014 at all three institutions, and again in August 2015 at RF2. The seals were on permanent display at their respective marine facilities and were fed and cared for under the guidelines of the individual facility. All Australian sea lions in the study were born as a part of an ongoing captive breeding program in Australian aquaria, while all fur seals came into captivity as juveniles, in poor health or injured, and were considered unsuitable for release. All individuals were in very good health during the study. This study was conducted under permits from Macquarie University ethics committee (ARA-2012_064) and Taronga ethics committee (4c/10/13).

We fitted seals with an accelerometer (CEFAS technology Ltd, Lowestoft, UK) set to record at +-8g, recording all accelerometer axes (X, Y, Z) at 1 sample per second (1Hz), and at a fast rate of 25 samples per second (25Hz). Behaviours we would expect to see in the wild were videoed, either elicited from

trainers with positive reinforcement or during seals 'free-time'. Videoed behaviours were matched to the accelerometry and scored by two investigators. Where behaviours scored by the investigators did not match, the video was reviewed and the behaviour recoded in agreement with both investigators. We defined 26 unique behaviours that we later grouped into four broader behavioural categories – foraging, travelling, grooming and resting, in three mediums – land, surface and underwater. Foraging behaviours consisted of searching for food and any prey handling. Grooming was any behaviour used in body maintenance or thermoregulation. Resting was any period of stillness, while travelling was any period of movement that was not foraging or grooming (full details of the method can be found in (Ladds *et al.* 2016c, Ladds *et al.* 2017b)).

Low resolution data (1Hz) were recorded for the duration of wild seal deployment so all low resolution data collected in captivity was kept for analysis. Fast rate data (20Hz) were set to record on the initiation of a dive (>1.5m). For some diving events the fast rate logger would continue recording at the completion of a dive, so fast rate data were collected both at the surface and while diving. We therefore segmented all captive data that occurred in the water for training (see below) of wild fast rate data. Further, as the captive data were recorded at a faster rate than the wild data, we used integration to reduce the sample rate of the captive data to 20Hz. We used the wet/dry switch on accelerometers to indicate when seals were in water or on land as this improves the ability of the models to predict behaviour. To determine the behavioural state of seals we used gradient boosting models (GBM) implemented in R using the package 'xgboost' (Chen *et al.* 2016). 'Super learners' produce the higher accuracies and lower variances than GBM when classifying seal behaviour. However, this method is computationally intensive, is complex to implement and only gives a very slight improvement on the accuracy overall (Chapter 5).

Captive data were split into one of three epochs (number of samples) for training the GBM. For high resolution data we used 13, 25 and 75 epochs, which correspond to 0.5, 1 and 3 seconds of data respectively. For low resolution data we used 7, 15 and 21 epochs which corresponded to 0.1, 0.25 and 0.33 minutes of data respectively. The number of behaviours available for training and testing meant we could not test epochs longer than this. Because of the uneven distribution of samples across the classes we used down-sampling to create even groups (Ladds *et al.* 2016c). The captive data were run over a grid of selection parameters (Ladds *et al.* 2017b) with the best parameters being chosen for implementation on the high and low frequency wild data. The raw data were then labelled with the results from the GBM model that produced the highest cross-validation (train) and out-of-sample (test) accuracies.

Behaviour events were labelled for the duration of deployment. Events were considered as different when either the location or the behaviour changed, and the change must have occurred for longer than 15 seconds otherwise the behaviour was kept the same as the previous behaviour. For wild data each event was classified with a probability of it being each behaviour, with the final behaviour labelled the behaviour that had the highest probability. To evaluate how well our models classified behaviours, we examined behaviours that were selected with less than 80% chance of occurring and extracted the behaviour with the next highest probability.

8.2.4. Time-energy budgets

Once data were labelled we estimated DEE by applying the energetic cost of each behaviour as a function of where it occurred (land, surface or underwater).

8.2.4.1. Resting energy expenditure

Energy consumption when resting in water is related to water temperature for postabsorptive female and subadult Australian and New Zealand fur seals (Ladds *et al.* 2017a). However, postprandial resting metabolic rate (RMR) for northern fur seal (*Callorhinus ursinus*) pups (Liwanag 2010) and juvenile South American fur seals (*Arctocephalus australis*) (Dassis *et al.* 2014) is 1.6 times the postabsorptive rate, lasting for around 3.5 hours. For simplicity, we assumed that seals were postabsorptive while on land, and postprandial in the water.

Resting in water:

$$\operatorname{EE}_{RMR}^{water}(l^{-1} O_2) = (0.02 + 0.02(\text{water temperature}) \times \text{duration}) \times 1.6$$
(8.1)

Unfortunately, no measure of juvenile Australian fur seals RMR on land has been made. In a separate study, we measured the standard metabolic rate (SMR) of a similarly sized New Zealand fur seal (47kg) over the course of a year (Ladds *et al.* 2017a), but these measurements were collected in water. As northern fur seal pups and southern sea lion subadults both had \sim 30% lower RMR on land than in water (Donohue *et al.* 2000, Dassis *et al.* 2012), we applied this assumption to our RMR estimation on land. In addition, as there was a seasonal effect on SMR for New Zealand fur seals (Ladds *et al.* 2017a), we calculated a summer and a winter metabolic rate (Eq. 8.2.1-2).

Winter RMR on land:

$$EE_{RMR}^{winter} (l^{-1} 0_2) = (0.41 \times 0.7) (duration)$$
(8.2.1)

Summer RMR on land:

$$EE_{RMR}^{summer}(l^{-1} O_2) = (0.53 \times 0.7)(duration)$$
(8.2.2)

8.2.4.2. <u>Activity energy expenditure</u>

Several attempts to derive the relationship between accelerometer output (ODBA or VeDBA) and energy expenditure have been made with mixed results (Chapter 7; Fahlman *et al.* 2008b, Volpov *et al.* 2015b, Jeanniard-du-Dot *et al.* 2016b). Recently this relationship has been demonstrated to fall into the "time-trap", whereby any relationship observed is a result of time being related to itself (Chapter 7; Halsey 2017a). Therefore, instead of deriving activity energy expenditure from DBA, we multiply the time an animal spent active (foraging or travelling) by the average energy expenditure (Ladds *et al.* 2016b). As yet, there have been no estimates of the cost of travelling on land for any pinniped, though experimentally the cost of movement on land is probably much greater than in water. In semi-aquatic water rats (*Hydromys chrysogaster*) the metabolic cost of running was around 13-40% more than swimming when moving at equal speeds, and for platypus (*Ornithorhynchus anatinus*), the cost of walking was 2.1 times the cost of swimming (Fish et al., 2001). As terrestrial locomotion in otariids is more similar to platypus than water rat, we assumed that the cost of movement on land is twice that in water. As activity compensates for some of the additional costs of cold water (Liwanag *et al.* 2009) we assumed that the energy expenditure for winter and summer was the same (Eq. 8.3.1).

Energy expended from activity (foraging and travelling):

$$EE_{active}^{water} (l^{-1} 0_2) = 1.43 (duration)$$
 (8.3.1)

$$EE_{active}^{land} (l^{-1} O_2) = (1.43 \times 2) (duration)$$
(8.3.2)

8.2.4.3. Grooming energy expenditure

The energy expended from grooming was estimated to be between 1.5 and 2 times the postabsorptive RMR and between 0.9 and 1.2 times postprandial RMR in northern fur seal pups depending on activity level (Liwanag 2010). Considering our model generally only labelled active grooming, we assumed that grooming had an energetic cost twice that of in-water RMR (Eq. 8.4.1-2).

Energy expended from grooming in winter in water:

$$EE_{groom}^{winter} (l^{-1} 0_2) = (0.41 \times 2) (duration)$$
 (8.4.1)

Energy expended from grooming in summer in water:

$$EE_{groom}^{summer} (l^{-1} 0_2) = (0.53 \times 2) (duration)$$
 (8.4.2)

For grooming on land seals were assumed to be postprandial so they were assumed to have the same energetic output as resting or slightly higher.

8.2.4.4. Overall energetics model

The final model was estimated by adding the estimated energy expenditure for each behavioural event over the course of a day as a function of location and time of year (Eq. 8.5.1-2).

Overall winter energetics model:

$$DEE (ml^{-1} O_2) = EE_{RMR}^{winter} + EE_{RMR}^{water} + \sum_{N=1}^{Ev_i} EE_{active}^{land} + \sum_{N=1}^{Ev_i} EE_{active}^{water} + \sum_{N=1}^{Ev_i} EE_{groom}^{winter}$$

$$(8.5.1)$$

Overall summer energetics model:

$$DEE (ml^{-1} O_2) = EE_{RMR}^{summer} + EE_{RMR}^{water} + \sum_{N=1}^{Ev_i} EE_{active}^{land} + \sum_{N=1}^{Ev_i} EE_{active}^{water} + \sum_{N=1}^{Ev_i} EE_{groom}^{summer}$$

$$(8.5.2)$$

8.3. Statistical analysis

We conducted a sensitivity analysis to investigate how changes in the proportion of time spent in different activities affects the overall DEE. The actual range of time spent in each behavioural category was used to calculate 500 simulated proportions, where each simulated proportion represented a day. The energetics model was applied to the simulated proportions, and the activities were grouped according to behaviour: active (travelling and foraging), grooming and resting; then by location: water

and land. The total DEE for the day was then plotted against the simulated proportions for each of the five categories.

We tested for significant differences on DEE with sex and season with individual seal identification as a random factor. We used a post-hoc general linear hypotheses and multiple comparisons test via the Tukey method with the function glht from the package "multcomp" (Hothorn et al., 2013). All analysis was completed in R (Version 3.1.3; R Core Development Team, 2015) and values are reported as mean \pm SD.

8.4. Results

8.4.1. Behaviour classification

The models classified captive data with very high accuracy, but the number of epochs used affected the overall accuracy (Table 8.1). The best low frequency model (1Hz) used 21 epochs, and the best high frequency model used 75 epochs, both of which had the highest training, testing and kappa scores for their category. As the 1Hz data classified behaviours so well (particularly for the test accuracy), and recorded for the duration of deployment, we chose to analyse only these data for our activity budgets.

Table 8.1 Cross-validation (train) and out-of-sample (test) accuracy for gradient boosting models (GBM) trained across a range of epochs using two datasets for all behaviours and for behaviour in water.

Behaviour	Hz	Epochs	Cross-validation accuracy	Out-of-sample accuracy	Kappa
ALL	1	7	78.3%	72.1%	71.1%
ALL	1	15	79.7%	86.8%	73.6%
ALL	1	21	80.8%	89.5%	73.6%
Water	20	13	63.2%	67.6%	68.6%
Water	20	25	72.4%	69.1%	63.2%
Water	20	75	82.7%	75.6%	76.9%

Some caution needs to be taken when interpreting the results from the activity budget analysis as the model is not always confident in its behavioural assignment. Therefore, the likelihood of belonging to a behavioural category was examined further to determine when the model may have been 'confused' between two categories. Epochs (time-windows) represent a snapshot in time of what the animal was doing. Our model assigns a probability of every epoch belonging to one of the four categories of behaviour. We then take the highest probability and assign that behaviour to that epoch. Most epochs (99%) were assigned to a behaviour with over an 80% probability. Figure 8.1 displays the scaled density distribution of the probability of belonging to the majority category (the class with the highest probability that a particular epoch belonged to). Figure 8.1A shows that when the model was uncertain that the

behaviour was foraging (less than chance) that the behaviour was generally predicted as travelling. The model almost never confused foraging and resting or grooming. When the model predicted travelling with a less than 50% chance, the behaviour with the next highest probability was foraging (Fig. 8.1B). Grooming (Fig. 8.1C) was rarely confused for other behaviours, but when this did occur the model predicted resting with the next greatest probability. Similarly, resting had few cases of confusion, and predicted foraging with the next highest probability.



Figure 8.1. Density plots representing the probability of an epoch belonging to a behavioural category. Each plot represents the probability of belonging to a behavioural category when the labelled category was predicted as the most likely class for that epoch.

Figure 8.2 shows an example of the output produced by the activity model. The time period shown is during the end of a foraging bout, transiting back to land and then a short period of the haul out. This figure serves to demonstrate the strengths and weaknesses of the GBM built from captive surrogates. The model was very good at predicting when the seal was resting, as this was when there was very little movement in the accelerometer. However, this also meant that dive ascent was classified as resting (Fig

8.2B). Grooming was also classified accurately, showing that it predominantly occurred immediately prior to or following a dive, or during the first hour or so after hauling out. Foraging and travelling were frequently misclassified by the model (Fig. 8A-B). The most common occurrence was that the descent of a dive was classified as foraging when it most likely should have been travelling. During long trips back to the haul out site, foraging also appeared periodically.

8.4.2. Summary dive parameters

Three seals from Seal Rocks and six seals from Lady Julia Percy were successfully recaptured with accelerometers still attached. All winter seals were longer in length (cm) than summer seals and were heavier (kg) than all but one of the summer seals (Table 8.2). Seals made between two and 45 diving trips lasting between 30 minutes and nine days. Juvenile fur seals tagged in winter (from Seal Rocks) made fewer and longer foraging trips than seals tagged in summer (from Lady Julia Percy). All other diving parameters were very similar between summer and winter seals (Table 8.2).

8.4.1. Time-energy budgets

The pressure sensor on the accelerometers on two of the wild seals (LJP_A10283 and LJP_A10284) failed for a portion of the deployment, therefore for those two the time spent underwater was significantly underestimated. Overall, seals spent as little as one third and up to nearly two thirds of their time on land (range 31-63%) with the remainder in the water (range 37-69%), where they spent most of that time being at the surface. Seals spent approximately half of the deployment resting (range 32-55%), predominantly on land (Fig. 8.3). Approximately 20% (range 13-25%) of seals time was used searching for food and another 22% (range 17-33%) was used for grooming. Seals travelled on average for 12% of the time (range 8-22%). Caution needs to be placed on the travelling estimate due to the confusion between travelling and foraging in the model (Fig. 8.3).

The average daily energy expenditure (DEE) for all seals in all locations was 25.67 ± 7.15 MJ d⁻¹ (range: 11.46 - 38.95 MJ d⁻¹) and adjusted for mass was 0.70 ± 0.23 MJ kg d⁻¹ (range: 0.15 - 1.19 MJ kg d⁻¹; Table 8.3). The maximum DEE was obtained from a seal that spent 12 hours continuously diving at sea (Fig. A 1). There were no significant differences in the DEE for females (25.57 ± 7.52 MJ d⁻¹) and males (24.54 ± 7.13 MJ d⁻¹; *post-boc* comparisons: Z = -0.51, p = 0.61) or for summer (25.65 ± 6.91 MJ d⁻¹) and winter deployments (24.23 ± 8.63 MJ d⁻¹; *post-boc* comparisons: Z = -0.78, p = 0.44).

Overall the most expensive behaviour was foraging making up over a third of the daily energetic budget (Table 8.3). However, it is likely that some of this time was travelling, and together these categories made up 60% of the energy budget. Resting on land made up a significant amount of the overall energetic budget (~16%) as this was the largest part of the activity budget (~50%). As seals spent so little time resting at sea, this only represented ~4% of the overall energetic budget. The least costly activity was underwater grooming (1.7%) which is likely to be a mistaken classification. Grooming made up around one fifth of the total budget, similar to resting, and most was grooming at the surface (14%). Travelling was almost one quarter of the energetic budget, though this is likely underestimated due to the confusion with foraging (Fig. 8.1).



Figure 8.2 An example foraging bout, transition and haul-out of a female juvenile Australian fur seal from Lady Julia Percy, Victoria, Australia. Panels show VeDBA, raw acceleration of the x, y and z axis, location (underwater, surface or land), behaviour state (travelling, resting, grooming, foraging) and depth. A) Shows the end of a foraging bout, transiting back to land and then a short period of the haul out. B) Shows three dives from the foraging bout.



Figure 8.3 Energy-activity budgets for nine juvenile Australian fur seals - six deployed in summer and three deployed in winter. Bars represent % of time spent in each type of activity over the duration of the deployment. Colours represent the location of the behaviour. Boxplots represent the minimum, 25%, median, 75% and maximum values of DEE (MJ d⁻¹) for the number of days presented in the top right hand corner of plots. *Five values were not plotted as they are outside the range of the z axis (DEE).

ID	Season	Sex	Mass (kg)	Number	Av. trip	Number	Av. max.	Av. dive	Max	Av.	Range	Average	Days
				of trips	duration	of dives	dive depth	duration	depth	DEE	DEE	Kleiber	
			Length (cm)		(days) (SD)		(m) (SD)	(s) (SD)	(m)	(MJ d ⁻¹)		multiple	
A09844	Summer	Female	35 107	14	0.3 (0.2)	1071	29.7 (21.3)	121.9 (79.4)	62	22.6 (4.5)	17.2-33.0	5.4	19
A09864	Summer	Female	30 110	4	1.2 (0.9)	1261	73.2 (23.0)	140.3 (39.9)	105	27.8 (6.7)	18.0-35.6	7.4	10
A10281	Summer	Female	42 119	12	0.8 (0.8)	2499	34.9 (9.5)	122.6 (38.3)	54	26.2 (7.8)	15.5-37.7	5.4	22
A10282	Summer	Female	30 107	6	0.8 (1.0)	1461	39.0 (8.9)	173.2 (70.9)	50	25.2 (6.5)	14.9-33.8	6.7	19
A10283	Summer	Male	34 110	45	0.1 (0.1)	1552	10.2 (7.8)	49.6 (36.2)	44	25.6 (3.0)	18.8-31.8	6.2	19
A10284	Summer	Female	35 108	21	0.2 (0.2)	1277	23.1 (12.7)	90.9 (47.7)	46	30.4 (6.8)	15.8-38.9	7.2	18
A09804	Winter	Male	41 130	2	5.8 (4.6)	2907	40.3 (31.8)	114.8 (85.6)	85	21.2 (10.)	12.3-37.1	4.5	8
A09867	Winter	Female	43 139	6	1.6 (0.4)	3113	33.2 (8.6)	128.6 (31.3)	63	24.7 (7.8)	12.0-37.3	5.0	16
A09869	Winter	Male	45 133	7	1.1 (0.7)	2158	51.4 (29.6)	121.9 (59.1)	81	25.2 (8.6)	11.4-37.0	5.0	14

Table 8.2 Summary statistics of daily energy expenditure (DEE MJ d⁻¹) and dive trip details for nine juvenile Australian fur seals. Seal details and mean, standard deviation, minimum and maximum DEE for the length of deployment.

Behaviour	Average	SD	Max	% total	
	DEE	DEE	DEE	DEE	
	(MJ d ⁻¹)				
Land	· • /				
Resting	4.15	2.09	9.58	16.1%	
Grooming	1.68	1.39	6.69	6.5%	
Active^	2.09	3.01	13.68	8.1%	
Surface					
Resting	0.64	0.57	2.86	2.5%	
Grooming	3.60	3.29	15.53	14.0%	
Foraging	3.42	2.67	10.77	13.3%	
Travelling	3.28	3.10	18.40	12.8%	
Underwater					
Resting	0.65	0.84	4.14	2.5%	
Grooming	0.44	0.63	3.05	1.7%	
Foraging	4.27	4.25	20.12	16.6%	
Travelling	1.49	1.49	6.48	5.8%	
Total					
Resting	5.44	3.50	9.58	19.6%	
Grooming	5.72	5.31	15.53	20.6%	
Foraging	10.20	10.78	20.12	36.7%	
Travelling	6.45	6.75	18.40	23.2%	

Table 8.3 Daily energy expenditure (DEE MJ d⁻¹) for different behaviours on land, at the surface and underwater for nine juvenile Australian fur seals.

^Active includes both foraging and travelling as we assumed any foraging that occurred on land was actually travelling.

The sensitivity analysis revealed that by varying the proportion of time spent in different behaviours in different locations that the DEE expected for a juvenile Australian fur seal varied between 24 and 36 MJ d⁻¹. Also, increasing the proportion of time active increased the overall DEE (Fig. 8.4A), while increasing the time resting decreased the overall DEE (Fig. 8.4C). This suggests that if total time spent active, or time spent resting was known, then the overall DEE can be predicted. Changes in time spent grooming did not affect the overall DEE (Fig 8.4B). Also, changes in the percentage of time spent on land or in the water does not affect the overall DEE (Fig. 8.4D-E).



Figure 8.4 Plot of 500 simulated points of total DEE against percentage of time spent: a) active (travelling and foraging); b) grooming; c) resting; d) in water; e) on land.

8.5. Discussion

This study developed time-energy budgets of wild fur seals across multiple foraging trips, using accelerometers recording at a low frequency (1Hz) validated from experiments with captive surrogates. Different activities come at different costs, therefore we hypothesised that energy expenditure can be estimated by measuring how much time an animals spends engaged in different activities (Maresh *et al.* 2015), and adjusting these costs for intrinsic and extrinsic factors (Ladds *et al.* 2017a). We first constructed models from machine learning that classified time-activity budgets of four typical fur seal behaviours (foraging, grooming, resting and travelling) with accelerometers recording at low and high resolution. We then built simple energetic models based on the amount of time spent in each activity multiplied by the cost of that activity adjusted for external (i.e. temperature) and internal (i.e. digestive state) influences where appropriate. As a result, we could construct time-energy budgets for wild juvenile Australian fur seals, from two locations in southern Australia, tracked over multiple foraging trips.

8.5.1. Behaviour segmenting with accelerometers and machine learning

Machine learning models trained with accelerometry data from captive animals reliably and accurately classified all four behaviour categories tested: foraging, grooming, resting and travelling. Models were trained with data from two sample rates, low (1Hz) and high (20Hz) resolution. We expected that when using a higher frequency the models would perform better at distinguishing between the different behaviour types (Halsey *et al.* 2009a). However, we found that the low resolution sampling regime produced much higher training and testing validation accuracies over a range of epochs (the number of samples on which summary data is calculated). The duration of the behaviour seemed to influence the result, as larger epochs tended to produce higher accuracies (Table 8.1). We surmise that the overall value of the summary statistic and its variation would be lower for long duration behaviours, i.e. that low energy, repetitive behaviours are easier to distinguish (Diosdado *et al.* 2015). Smaller window sizes are more likely to pick up irregular movements of the animal that arise from short duration high energy activities (such as burst attacks on prey) which we rarely saw in the captive experiments. Instead, foraging was defined by handling of dead prey and actively searching the bottom of the pool for food hidden in the substrate (Ladds *et al.* 2016c). From this definition foraging became the most difficult behaviour category for the model to classify (Fig. 8.1).

Accelerometers deployed on wild seals were set to record continuously at a low resolution (1Hz) and at a high resolution (20Hz) during dives (>1.5m). Considering the success of the low resolution models from captive animals and that the data from the wild was continuous, we focussed our analysis on this data only. The trained model based on captive animals could predict the behaviour of the wild seals, with some anomalies. A the macro level, the activity budgets matched what we expected to see from wild seals (Battaile *et al.* 2015), where they spent most of their time resting (~45%), and the rest of their time was split evenly between other activities – grooming (~22%), foraging (~20%) and travelling (~12%; Fig. 8.2).

On the micro level, the model classified some events incorrectly, as foraging and travelling were often confused (Fig. 8.1). The confusion in the model came primarily from two sources – classifying the descent of the dive as foraging, when it is more likely travelling, and from identifying bouts of foraging during long bouts of surface swimming transiting back to the colony. Distinguishing foraging behaviours for other species has also been difficult. For example, the peck and attack of plovers could not be classified using supervised machine learning (Bom *et al.* 2014). While the distinction between foraging and travelling needs to be refined in future models, the combined time that the seals spent in the two behaviours is likely accurate.

8.5.2. Time-energy budgets

8.5.2.1. Grooming

Seals spent approximately 25% of their time-at-sea grooming, compared to northern fur seals that spend around \sim 30% of their time at sea rolling at the surface, and another 9% in other grooming activities (Battaile *et al.* 2015). The total energetic cost of daily grooming overall was not different to resting, despite

having up to twice the energy demand (Liwanag 2010). On average seals spend 3.60 MJ d⁻¹ grooming at the surface of the water and 1.68 MJ d⁻¹ grooming on land, contributing \sim 20% to the overall energy budget. Grooming is used for general body maintenance and can offset some of the costs of thermoregulation (Liwanag 2010, Iwata *et al.* 2013). To thermoregulate at sea, seals float with either their hind flippers (jughandling) or their fore flippers (sailing) in the air, enabling perfusion of the flippers in cool and warm water (Liwanag 2010). This allows heat to escape their hairless flippers in warm water, or to avoid heat loss in cold water (Bartholomew and Wilke 1956).

Grooming in water generally occurred prior to or at the completion of a dive (Fig. 8.1). While diving, fur becomes compressed, reducing its effectiveness to provide insulation, warmth and buoyancy (Fish *et al.* 2002, Dickerson *et al.* 2012, Iwata *et al.* 2013), and to counteract this fur seals roll at the surface while rubbing their body with their fore flippers in order to trap air bubbles into their pelage (Liwanag 2010, Battaile *et al.* 2015). This aids in maintaining positive buoyancy which helps with energy saving diving techniques (discussed below; Fish *et al.* 2002) and to raise the metabolic rate in cold water (Liwanag 2010). General body maintenance, such as rubbing whiskers, can occur after consuming large prey items. Wild polar bears (*Ursus maritimus*) have been observed to clean regularly while consuming prey where they pause eating at regular intervals to rinse and lick their fore paws and face (Stirling 1974). Fur seals also spent a lot of time grooming on land (~15% of all land activity) using their flippers and occasionally their teeth to maintain their fur.

8.5.2.2. Resting

Juvenile fur seals spent around half of their time resting, which contributed around ~20% to their overall energetic budget (Table 8.3). Due to the large cost of travelling and foraging, seals must use long haul out periods to rest and recuperate. This is particularly true of juveniles who have an additional cost of growth (Burns *et al.* 2004, Richmond *et al.* 2006, Ladds *et al.* 2016b), and use this time for reintegrating tissue and laying down fat (Kirsch *et al.* 2000). Juvenile fur seals on average spent 72% of their time resting on land, which was ~16% of their overall activity budget. Fur seals tagged in summer spent 10% longer on land than seals in winter, but on average expended similar amounts of energy. This suggests that in summer seals need longer to recuperate than winter seals. During long periods of time ashore, seals generally remain motionless for energy conservation while fasting (Stirling 1971). During the breeding season, adult male northern and subantarctic fur seals spend >90% of observed time (during the day only) motionless, either sitting or lying (Stirling 1971, Bester and Rossouw 1994). During the breeding season, New Zealand fur seal adult males and females spend 60-70% of their daylight hours lying down (Crawley *et al.* 1977).

The fur seals in this study spent ~12% of their time at sea resting, ~2% of this time underwater. Some phocid seals rest underwater (Mitani *et al.* 2009), though it is unlikely the fur seals use this strategy as their dives were generally no longer than a few minutes duration (Table 8.2). Instead, resting underwater can be explained by the model classifying the ascent part of the dive as resting. Northern fur seals and Antarctic fur seals (*Arctocephalus gazella*) rest for less than 10% of their time at sea (Battaile *et al.* 2015, Jeanniard-du-Dot *et al.* 2016b), which is similar to what we found here. Fur seals, in particular juveniles (Fowler *et al.* 2006), have shorter trip durations than phocids, meaning that they need less time resting at-sea (Maresh *et al.* 2015).

Long periods of gliding on the ascent part of the dive, likely results from the seals being positively buoyant. During underwater rest or glide periods metabolic rate is at or lower than RMR (Williams 2001, Fahlman *et al.* 2008a). Diving seals reduce their metabolic rate in order to conserve their on board oxygen stores (Ponganis *et al.* 2011) and to do so, make behavioural compensations such as gliding (Williams 2001, Williams *et al.* 2004b). Weddell seals (*Leptonychotes weddelli*) spend 40% of the final part of a dive gliding with intermittent strokes that reduces their overall energy expenditure (Fuiman *et al.* 2007). Therefore, classifying this part of the dive as resting, thus having a lower metabolic rate associated, would strengthen the validity of the models.

8.5.2.3. Movement (Foraging and Travelling)

Derivation of our energetic budget distinguishes between two sedentary behaviours (resting and grooming) and active behaviours (foraging and travelling). While there has been evidence that accelerometers can be used to measure the active behaviours (Jeanniard-du-Dot *et al.* 2016b), a recent commentary has revealed that these relationships are in fact confounded by time (Halsey 2017a). As a result, we chose to estimate active energy expenditure as a function of time, where we multiplied an estimate of average energy expended while active (measured via respirometry in captivity (Ladds *et al.* 2016b)) by the amount of time spent active at sea. This approach made two main assumptions; that seals were postabsorptive at sea and that the cost of foraging and travelling were equivalent. Assuming seals are postabsorptive at-sea (and postprandial on land) is a valid assumption as there is evidence that seals partially delay digestion while diving (Rosen and Trites 1997, Rosen *et al.* 2015). The cost of foraging and travelling in this study were unable to be separated because of the models' tendency to confuse the behaviours (Fig. 8.1). While this did not happen frequently, the two behaviours were inextricably linked due to the common movement of the behaviour (Ladds *et al.* 2017b), and due to this commonality of movement, it was assumed that the energetic cost would be similar.

A more difficult estimate to make was for energy expended from locomotion on land as it is yet to be measured for otariids. Movement on land is likely far more costly than in water because seals morphology has adapted them for efficiency in the ocean (Beentjes 1990). In a commercial kill of northern fur seals animals that were exposed to prolonged stress of being chased on land would often die of heat stress despite temperatures of $<10^{\circ}$ (Bartholomew and Wilke 1956) indicating that movement on land for fur seals is energetically costly. Further experimental evidence for the higher energetic cost of movement on land for semi-aquatic animals comes from platypus and water rats, where both species had a significant increase on metabolic rate walking on land as opposed to swimming at a similar velocity (Fish and Baudinette 1999, Fish *et al.* 2001). Based on this knowledge we assumed that the cost of travelling on land was twice as costly for fur seals as swimming in water. As a result, the average DEE of activity on land was 2.09 MJ kg⁻¹, which was ~8% of the overall energetic budget. Given the assumed high cost of travelling on land and that travelling on land represented only ~4% of the overall activity budget, juvenile fur seals likely minimise the time spent active on land to save energy for foraging.

Juvenile Australian fur seals spent around half of their time in water (36-69%). During the breeding season adult female Australian fur seals spend around 75% of their time in the water (Arnould and Hindell 2001a) while pups are only in the water for around 29% of their time (Spence-Bailey *et al.* 2007). Of the time juveniles are in the water, approximately 56% (35-62%) is spent foraging and travelling, which contributes to most (~60%) of their DEE (Fig. 7.3, Table 7.4). The large cost of travelling and foraging is predominantly from the mechanical power from strokes during swimming. This is likely because during a dive, seals use a range of behavioural compensatory techniques that lower metabolic rate to remain below the water for longer (Davis and Williams 2012).

8.5.3. Implications for fitness and survival

Overall, juveniles had an average DEE that was 5.8 times the predicted basal metabolic rate, which was higher than estimated energy expenditure measured from adult female Antarctic fur seals and northern fur seals (4.7 times BMR; Jeanniard-du-Dot *et al.* 2016b) and slightly higher than adult female Californian sea lions (*Zalophus californianus californianus*) (5.2 times BMR; Ponganis *et al.* 1997). This higher cost was expected because of the additional cost of growth for young fur seals (Burns *et al.* 2004, Ladds *et al.* 2016b) which result in compensatory techniques such as shorter diving durations and longer haul-outs than adults. DEE did not differ for sex or for season (winter vs. summer). Sex differences were not expected for juveniles, as any differences in diving abilities (Fowler *et al.* 2006), physiological parameters (Burns *et al.* 2004, Verrier *et al.* 2011) or survival (Beauplet *et al.* 2005) have been attributed to age or size rather than sex (Weise and Costa 2007). Also, the lack of difference between seasons can be attributed to behavioural adaptations, where in summer, when seals would be expected to expend more energy, seals utilise haul outs more often to conserve energy.

Surface travelling and underwater foraging were the most energetically expensive behaviours for seals while at sea (Table 8.3). Therefore juveniles are likely to conserve energy by reducing the amount of time searching food (Verrier et al. 2011), and by engaging in energy saving foraging strategies (Maresh et al. 2015). This was evident here by the large proportion of time fur seals spent hauled out, the short trip durations in comparison to adults and gliding during dives. Australian fur seal pups nine months of age did not have the diving capability of adults, having significantly lower mean and maximum dive depths and durations than adult females (Arnould and Hindell 2001a, Spence-Bailey et al. 2007). Australian fur seal juveniles demonstrated dive depths and durations that were greater than pups, but they still had not reached adult levels (Table 8.2; Arnould and Hindell 2001a). Similarly, juvenile New Zealand fur seals and Australian sea lions dive for shorter durations and to shallower depths than adults of the same species (Fowler et al. 2006, Page et al. 2006). This is likely because there are significant constraints placed on juvenile fur seals when foraging and travelling. Higher mass-specific metabolic rates (Fowler et al. 2007b, Ladds et al. 2016b) and lower oxygen stores (Burns et al. 2004) restrict the duration for which juveniles otariids can forage. Coupled with the inexperience of learning how to dive and forage (Merrick and Loughlin 1997, Leung et al. 2013) juveniles are likely working harder, on a mass-specific basis, to sustain their energetic needs than adults.

8.5.4. Conclusion

This study demonstrated that daily time-energy budgets for free-living juvenile Australian fur seals can be developed from low resolution accelerometry data. Previous studies interpreting the foraging behaviour (Battaile *et al.* 2015) or energy expenditure (Jeanniard-du-Dot *et al.* 2016b) of wild fur seals have used high resolution data, at a cost of space and battery power from the device. Fine tuning of the model presented here would allow for detailed time-energy budget on a minute or hourly basis, but the current methodology provides a representative estimate of daily time-energy budgets for wild fur seals. Juvenile fur seals appear to be working harder than their adult counterparts. This likely extends from the relative novelty of diving and foraging and the additional cost of growth they sustain. The costs appear to be constant across sexes and seasons. Juvenile fur seals change their behavioural strategies to compensate for the higher metabolic rate they likely have in summer. Species fitness and survival is dependent animals achieving balance between energy intake and energy expenditure.

Global climate change will bring unintended consequences to the oceans upon which seals rely on for food. Warming waters and strengthening currents will likely change the availability and distribution of once reliable prey sources. Monitoring how seals respond to these changes requires an understanding of how their energy needs change over time. Accelerometers afford an opportunity to investigate not only how seals expend their energy (this Chapter), but can also be used as a tool for energy intake (Volpov *et al.* 2015a). Accelerometers allow us to monitor how seals change their activity patterns to accommodate energy losses and gains, and to quantify their impacts in the face of global ocean changes.

Chapter 9

Conclusions and future directions

At the broadest level in biology we aim to understand animal fitness - how they survive to reproduce while interacting with their environment (Orr 2009). Animals have different energetic requirements based on the time of year, their age and reproductive status, the surrounding environment and their diet (Nagy 1994). While some of these aspects of energetic variability may remain constant, rapid environmental change makes it difficult to predict how an animal's needs may change and if they have the capacity to adapt (Simmonds and Isaac 2007, Boyles *et al.* 2011). The waters of South-East Australia are warming much faster than the global average (Ridgway and Hill 2012) and this area is home to three important marine predators; the New Zealand fur seal, the Australian fur seal and the endangered Australian sea lion. These species have a number of complementary and contrasting characteristics that make them interesting and useful to examine their energetics in depth, in order to understand their potential responses to a changing environment. All three species have overlapping geographic ranges, but utilise different foraging methods in order to find prey (Page *et al.* 2005a, Kirkwood *et al.* 2006, Page *et al.* 2006, Arnould and Kirkwood 2007, Lowther *et al.* 2011, Shaughnessy *et al.* 2011, Lowther *et al.* 2013) and their current population trajectories are different following recovery from exploitation (McIntosh *et al.* 2012, McIntosh *et al.* 2014, Shaughnessy *et al.* 2014).

However, estimating the energetic needs of populations of wild animals is challenging (Halsey 2011). In this thesis I investigated the energetic demands of fur seals and sea lions, and completed a rigorous assessment of the utility of a widely-used bio-logger – the accelerometer – to measure the behaviour and energetics of fur seals and sea lions. Bio-logging affords new opportunities to measure animal activity and energetic expenditure, but only if accurately calibrated (Halsey *et al.* 2009a). Calibrating these tools can be difficult, particularly for large, wide ranging animals. I validated and tested the utility of using accelerometers to measure animal behaviour and energetics with an underutilised resource, captive animals. The three species of interest are commonly housed in marine facilities in Australia, either as a part of an ongoing captive breeding program for the endangered Australian sea lion, or as sick or injured stranded fur seals that have been brought in for care and are unsuitable for release. These animals provided an opportunity to validate accelerometers as a tool to measure behaviour and energetics of fur seals. The captive animals also afforded a unique opportunity to understand how different foraging behaviours used in the same environment may impact physiology and survival (Costa *et al.* 2004, Arnould and Costa 2006). First it was necessary to acquire a basic understanding of their physiology.

In Chapter 2 I measured the standard metabolic rate (SMR; a proxy for resting metabolic rate) of 12 captive fur seals and sea lions using respirometry. I demonstrated that SMR varied across species, age classes and sexes and, for fur seals, was influenced by annual cycles. Fur seals and sea lions responded very differently to time of year, with fur seals showing predictable changes in metabolic rate in response

to the moult, while sea lions showed no predictable metabolic rate changes. Both fur seal and sea lion females had higher metabolic rates than males, but there were no species differences in average metabolic rate when comparing animals of the same sex. For both species metabolic rate responded to changes in water temperature, with warmer temperatures reducing the metabolic rate of sea lions but stimulating an increase in fur seal metabolic rate. This information is important for estimating the energy expenditure of wild seals as accelerometers are not able to measure this type of variation (Dalton *et al.* 2014b).

I extended the results of Chapter 2 in Chapter 3 by investigating how different factors influenced active metabolic rate (AMR) and the cost of transport (COT). I ran experimental trials in which fur seals and sea lions swam between two submerged target poles for varying durations before surfacing in a respirometry hood. Speed and the duration of submerged swimming influenced overall AMR and COT. Despite not diving to great depths I found that the longer the period of submerged swimming the lower the metabolic rate became, which was consistent with the animals demonstrating a dive response (Kooyman *et al.* 1981). For the fur seals and sea lions in this study, the cost of transport increased exponentially with speed, and the optimal swim speed lay between 1.5 and 2m/s. This is the most commonly reported swimming speed of wild fur seals and sea lions, thus validating our measurement approach (Ponganis *et al.* 1992). I observed that the cost of activity was much higher for subadult and adult female fur seals and sea lions than adult males, but there were no species differences when comparing animals of the same age class and sex. This indicated that females and younger seals were potentially more vulnerable to environmental changes than other cohorts.

Different activities have different energetic costs, so accurately estimating the energy expenditure of wild animals requires knowledge of the amount of time spent in different activities, as well as the cost of that activity. Therefore, in Chapters 4 and 5 I used captive animals to validate the use of accelerometers to classify behaviour automatically using machine learning. We attached accelerometers to 12 fur seals and sea lions varying in size, age, species and sex and were able to automatically classify four main categories of behaviour from the seals: foraging, travelling, grooming and resting. In these two chapters I explored the different options available for training and testing data using different machine learning methods. I found that model selection, the number of behaviours and time windows used to segment the data heavily influence the prediction accuracy. For this type of data fewer categories and smaller time windows improved the accuracy results. I also discovered that to make the model generally applicable to other animals, some characteristics of the animal (sex, species, mass) and the medium of the behaviour (land, surface, underwater) must be included in the model.

In Chapter 6 I explored the utility of accelerometers as a proxy for measuring energy expenditure derived from activity, and sought to understand how this changes for different species, sexes and age classes. I found that accelerometers were a useful proxy for measuring energy expenditure over a single submergence time when calibrated for dynamic body acceleration (DBA) or stroke rate. DBA explained more of the variation in total energy expenditure than did stroke rate or submergence time, likely because it considers the unusual manoeuvres that seals use underwater. However, neither DBA nor stroke rate could predict the rate of energy expenditure, as it predictably declined with increased submergence time.

While submergence time on its own predicted energy expenditure, using accelerometers for estimating energy of wild populations is essential, as the accelerometer can concurrently identify the different behaviours used by fur seals and sea lions and their duration (Chapter 4 and 5). This approach makes it possible to estimate daily energy expenditure which provide incredible insights into animal ecology.

Chapter 7 brings together all the new information and insights from the previous chapters. To test the utility of using accelerometers for making time-energy budget of free living seals, I deployed devices on wild juvenile Australian fur seals. Juveniles were an ideal test subject as they were small enough to catch and restrain, and they predictably return to the same haul out for recapture. I collected information from nine seals in two locations in different seasons (winter and summer). Accelerometers continuously recorded low resolution data but recorded high resolution data for dives and some surface time. Using models developed in Chapters 4 and 5, I tested the classification accuracy of both high and low resolution data and found that low resolution data sampled on long time windows produced the highest accuracies for classifying four behaviours: foraging, travelling, grooming and resting. I applied these models to the data collected from wild seals and create a time-activity budget spanning days to weeks that included multiple foraging trips.

Next I applied the results of my captive trials (Chapters 1, 2 and 6) to the different activities extracted from the accelerometers to create time-energy budgets for the juvenile fur seals. I found that the fur seals spent the same amount of energy in summer and winter, although in summer seals spent more time resting, possibly to accommodate the higher metabolic rates they experience in summer. I also found no difference in the amount of energy expended by males and females, presumably as a result of being morphometrically similar and non-reproductive. Juveniles had a slightly higher average DEE than adult female fur seals, likely attributable to the cost of growth and foraging inexperience. Most importantly, the results from this chapter demonstrate that time-energy budgets of wild fur seals can be estimated using accelerometers validated through captive experiments.

While this thesis provides a thorough investigation of fur seal and sea lion physiology and behaviour, it is by no means exhaustive. A big advantage of captive experiments is that experimental conditions can be controlled and manipulated, though they are often limited by sample size. Continued captive experiments with animals from categories of animals not investigated in this thesis or elsewhere (i.e. adult female New Zealand fur seals) could fill these gaps.

Sea-surface temperature rise is a potentially problematic consequence of climate change for fur seals and sea lions (Learmonth *et al.* 2006). While I explored how water temperature affected the metabolic rate of some fur seals and sea lions in captivity, this was for a small range of temperatures with only a few animals, and I did not consider the effect of activity. This topic deserves more attention, as it is particularly intriguing that fur seals and sea lions respond differently to warming temperatures. This not only has consequences for their fitness in the wild, but it is also incredibly interesting in terms of their ecology and evolution.

Understanding how animals respond to environmental challenges will be crucial with the rapid onset of climate change (Simmonds and Isaac 2007). Accelerometers offer a potential solution for monitoring some of these changes, but only if linked to energy acquisition as well as energy expenditure. Accelerometers have the potential to identify prey capture in seals, but only if head mounted (Volpov *et al.* 2015a). Therefore, future studies of wild seals should seek to place accelerometers on the head to identify prey capture and on the mid-line of the body to estimate energy expenditure and develop time-energy budgets. Deployed over time the combination of these methods would allow for estimation of foraging budgets to see if seals can maintain a balance between energy gain and energy expenditure. If food sources become increasingly sparse, then it would be expected that seals would increase their foraging time with less successful prey captures, a potentially easy theory to test with two accelerometers.

The goal of this thesis was to derive time-energy budgets for wild fur seals and sea lions using biologgers. I have demonstrated that captive surrogates of a species are an excellent resource for developing models to be applied to their wild counterparts. This is demonstrated by the interesting and important findings I have made about fur seal and sea lion behavioural and physiological adaptations to their environment. These results have provided crucial information to further our understanding of how wild fur seals and sea lions will adapt to a changing environment.
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Appendices

Appendix 1. Parameter grid search variables.

Random forests were tuned over a grid of four parameter variables: mtry, ntree, max depth and nBins. Mtry is the number of variables randomly sampled to be tested for the best split at each split point in the tree, while ntree is the total number of trees grown. Max depth is the maximum depth to grow the trees, shallower trees (lower numbers) lead to simpler models and can control over fitting. Deeper trees will allow for more interactions between variables to be found. To find the optimal nBins, H20 builds a histogram to assess the splitting points, a histogram of this many bins is built and the best point selected as the split. As with max depth less bins (lower numbers) leads to a simpler model and can control over fitting.

For stochastic gradient boosting (GBM), max depth and nBins are as per random forest. The idea behind a GBM is for each successive tree is built from the error of the previous tree. ntree controls how many of these iterations occur and the learning rate controls how fast the model learns the error. Setting of these two parameters can be viewed as a compromise between fast learning (low iterations, high learning rate) and good generalisation (high iterations and low learning rate). The sample rate used by the GBM subsamples the data without replacement at each boosting round and each model is trained on this subsample.

For logistic regression (LR) and the super learner (SL) two parameters were used: alpha and lambda. There are two forms of regularisation used in logistic regression, the L1 and L2 norms. Alpha sets the form of regularisation used, where alpha of 0 is L1 regularisation and an alpha of 1 is L2 regularisation. Whereas with an alpha of 0.5 the model uses both forms of regularisation each weighted by half. Lambda is the overall weight on the regularisation penalty. Higher lambda values will shrink coefficients in the regression towards zero to provide more regularised (simpler) models. The SL used two lambda values per alpha: the best preforming lambda, and the lambda value from the most regularised model such its cross-validated error is within one standard deviation of the minimum cross-validation error.

Appendix 2. Final ethics approval

4/21/2017

Gmail - Outcome of 16 February 2017 AEC Meeting - Final Report - ARA 2012/064

M Gmail

Monique Ladds <monique.ladds@gmail.com>

Outcome of 16 February 2017 AEC Meeting - Final Report - ARA 2012/064 3 messages

Animal Ethics <animal.ethics@mq.edu.au>

Fri, Feb 17, 2017 at 4:04 PM

To: Robert Harcourt <robert.harcourt@mq.edu.au> Cc: Monique Ladds <monique.ladds@mq.edu.au>, David Slip <david.slip@mq.edu.au>, Mr Mark Ryan <mark.ryan@underwaterworld.com.au>, Robyn Gentle <robyn.gentle@mq.edu.au>, Robby Miller <robert.miller@mq.edu.au>

Dear Prof. Harcourt,

Your Final Report for the above project was considered and accepted at the Animal Ethics Committee meeting of 16 February 2017.

2012/064 Professor Robert Harcourt

Investigating the energetics and diets of seals

(Biological Sciences)

Original number of animals approved: 27

Total number of animals used: 18

Thank you and all the best for your future research endeavours.

This email serves as official notification of the AEC decision. Please keep a copy for your records. Should you have any queries or require clarification, please contact the AEC Secretariat.

Regards,

A/Professor Jennifer Cornish Chair, AEC Animal Ethics

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Table A 1 Description and acceleration profile for 26 unique behaviours recorded. Black line -x axis acceleration; grey line -y axis acceleration; orange line -z axis acceleration.

Behaviour Behaviour description

RESTING





Seconds



Seconds



Behaviour Behaviour description

TRAVELLING



Seconds



Seconds



Seconds



Seconds



Table A 2 Sensitivity and specificity from training data with and without feature statistics for four machine learning models. Random forest (RF), logistic regression (LR), stochastic gradient boosting (GBM) and support vector machines (SVM) with four different kernels.

	Features	FALSE		TRUE	
Model	Behaviour	Sensitivity	Specificity	Sensitivity	Specificity
RF	foraging	0.640	0.891	0.711	0.930
	grooming	0.860	0.934	0.938	0.945
	resting	0.861	0.950	0.873	0.961
	travelling	0.642	0.893	0.699	0.905
LR	foraging	0.593	0.830	0.606	0.916
	grooming	0.714	0.892	0.803	0.902
	resting	0.856	0.901	0.829	0.921
	travelling	0.386	0.893	0.616	0.879
SVM Linear	foraging	0.638	0.801	0.596	0.917
	grooming	0.733	0.904	0.808	0.907
	resting	0.859	0.909	0.836	0.927
	travelling	0.339	0.909	0.621	0.868
SVM Polynomial	foraging	0.689	0.863	0.707	0.920
	grooming	0.776	0.943	0.904	0.938
	resting	0.868	0.919	0.863	0.953
	travelling	0.571	0.909	0.679	0.907
SVM Radial	foraging	0.676	0.861	0.697	0.925
	grooming	0.764	0.943	0.916	0.936
	resting	0.851	0.919	0.870	0.952
	travelling	0.559	0.894	0.679	0.907
SVM Sigmoid	foraging	0.679	0.818	0.592	0.918
	grooming	0.712	0.904	0.770	0.905
	resting	0.892	0.887	0.852	0.915
	travelling	0.320	0.924	0.598	0.866
GBM	foraging	0.641	0.885	0.752	0.920
	grooming	0.822	0.939	0.920	0.946
	resting	0.852	0.945	0.850	0.962
	travelling	0.632	0.881	0.710	0.916
Table A 3 Accuracy summaries and sample size used to train and test for four machine learning models. Models were tested across four different size epochs and with four and six behavioural categories. Statistics reported are: cross validation (training) accuracy and 95% confidence interval; out-of-sample (testing) accuracy and standard deviation (SD); the Kappa statistic and the proportion improvement made by the SL compared to other models.

				Cross	Cross validation	Cross validation	Out-of-			
		Behavioural		validation	accuracy lower	accuracy upper	sample	Out-of-sample		SL
Model	Epochs	categories	n	accuracy	bound	bound	accuracy	accuracy SD	Kappa	improvement
SL				0.834	0.810	0.856	0.835	0.007	0.779	
RF		4	1055	0.823	0.798	0.845	0.832	0.025	0.764	0.011
GBM		4	1055	0.820	0.795	0.843	0.834	0.022	0.760	0.014
LR	75			0.770	0.743	0.795	0.769	0.009	0.693	0.064
SL	/3			0.720	0.662	0.774	0.705	0.011	0.664	
RF		(2(1	0.678	0.618	0.734	0.697	0.034	0.613	0.042
GBM		0	201	0.709	0.650	0.763	0.712	0.030	0.651	0.011
LR				0.632	0.571	0.691	0.651	0.017	0.558	0.088
SL				0.834	0.820	0.847	0.836	0.004	0.778	
RF		4	2002	0.826	0.812	0.839	0.833	0.013	0.767	0.008
GBM		4	2962	0.834	0.820	0.847	0.834	0.013	0.779	0.000
LR	25			0.744	0.728	0.760	0.753	0.004	0.659	0.089
SL	23			0.730	0.700	0.759	0.721	0.014	0.676	
RF		6	806	0.723	0.693	0.752	0.699	0.044	0.668	0.007
GBM		0	890	0.720	0.689	0.749	0.712	0.039	0.664	0.010
LR				0.650	0.617	0.681	0.661	0.012	0.579	0.080
SL				0.851	0.841	0.860	0.841	0.002	0.801	
RF	13	4	5899	0.844	0.834	0.853	0.834	0.006	0.792	0.007
GBM				0.847	0.837	0.856	0.838	0.007	0.796	0.004

Table A 3 continued.

Model	Epochs	Behavioural categories	n	Cross validation accuracy	Cross validation accuracy lower bound	Cross validation accuracy upper bound	Out-of- sample accuracy	Out-of-sample accuracy SD	Kappa	SL improvement											
LR				0.751	0.740	0.762	0.750	0.003	0.668	0.100											
SL				0.736	0.715	0.757	0.741	0.009	0.684												
RF		6	1783	0.727	0.706	0.747	0.735	0.028	0.672	0.010											
GBM		0	1703	0.734	0.712	0.754	0.740	0.031	0.680	0.003											
LR				0.651	0.628	0.673	0.658	0.005	0.581	0.086											
SL				0.842	0.835	0.849	0.847	0.002	0.790												
RF		4	10709	0.837	0.830	0.844	0.842	0.007	0.782	0.006											
GBM		+	10/02	10707	10707	10707	10707	10707	10/07	10/07	10707	10707	10/07	10107	0.840	0.833	0.847	0.843	0.006	0.786	0.003
LR	7			0.741	0.733	0.750	0.750	0.002	0.655	0.101											
SL	/			0.716	0.695	0.737	0.724	0.005	0.659												
RF		(1900	0.705	0.683	0.726	0.718	0.017	0.646	0.011											
GBM		0	1800	0.717	0.696	0.738	0.719	0.016	0.661	-0.001											
LR				0.645	0.622	0.667	0.650	0.005	0.574	0.071											

			4 Feature	es						6 Feati	ires				
Epochs			Forage	Groom	Rest	Travel	Epochs			Feed	Forage	Groom	Rest	Thrash	Travel
	SL	sensitivity	0.807	0.873	0.874	0.785		CT.	sensitivity	0.511	0.689	0.733	0.911	0.861	0.644
		specificity	0.936	0.965	0.959	0.917		SL	specificity	0.917	0.926	0.968	0.949	0.969	0.935
		sensitivity	0.759	0.873	0.870	0.793			sensitivity	0.489	0.489	0.733	0.911	0.806	0.667
	RF	specificity	0.940	0.967	0.955	0.901		RF	specificity	0.912	0.931	0.935	0.935	0.960	0.940
75		sensitivity	0.789	0.869	0.863	0.763	75	0.001	sensitivity	0.489	0.689	0.733	0.889	0.889	0.600
	GBM	specificity	0.932	0.963	0.952	0.912		GBM	specificity	0.926	0.917	0.968	0.949	0.947	0.944
	LR	sensitivity	0.785	0.800	0.837	0.659			sensitivity	0.378	0.489	0.711	0.844	0.806	0.600
		specificity	0.908	0.931	0.927	0.926		LK	specificity	0.907	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	0.973	0.921		
		sensitivity	0.776	0.907	0.875	0.779	25	01	sensitivity	0.527	0.767	0.873	0.773	0.781	0.660
	SL	specificity	0.938	0.970	0.962	0.908		SL	specificity	0.926	0.916	0.972	0.981	0.961	0.920
	ЪГ	sensitivity	0.761	0.903	0.879	0.761		DE	sensitivity	0.527	0.747	0.873	0.800	0.808	0.587
25	KF	specificity	0.935	0.970	0.956	0.906		KF	specificity	0.929	0.912	0.962	0.969	0.949	0.946
25	ODM	sensitivity	0.788	0.917	0.875	0.759		ODM	sensitivity	0.540	0.747	0.867	0.773	0.788	0.607
	GBM	specificity	0.935	0.966	0.962	0.916		GBM	specificity	0.929	0.912	0.965	0.973	0.961	0.924
	тD	sensitivity	0.679	0.805	0.861	0.635		τD	sensitivity	0.400	0.720	0.787	0.773	0.705	0.513
	LR	specificity	0.916	0.933	0.893	0.917		LK	specificity	0.933	0.895	0.932	0.934	0.961	0.924

 Table A 4 Sensitivity and specificity of each behaviour category for all model combinations tested.
 SL – super learner, RF – random forest, GBM – stochastic gradient boosting, LR – logistic regression.

			4 Feature	es						6 Feati	ures				
Epochs			Forage	Groom	Rest	Travel	Epochs			Feed	Forage	Groom	Rest	Thrash	Travel
	SL	sensitivity	0.832	0.922	0.881	0.773		CT.	sensitivity	0.617	0.730	0.877	0.807	0.739	0.650
		specificity	0.936	0.970	0.964	0.930		SL	specificity	0.907	0.941	0.976	0.965	0.971	0.924
	DE	sensitivity	0.813	0.921	0.875	0.772		DE	sensitivity	0.597	0.730	0.877	0.823	0.746	0.590
12	KF	specificity	0.932	0.967	0.964	0.928	12	KF	specificity	0.909	0.939	0.972	0.956	0.958	0.939
13	0.0.1	sensitivity	0.829	0.927	0.870	0.766	13	CDM	sensitivity	0.590	0.750	0.870	0.803	0.753	0.637
	GBM	specificity	0.934	0.966	0.965	0.930	GBW	specificity	0.911	0.939	0.973	0.962	0.963	0.931	
	LR	sensitivity	0.733	0.798	0.849	0.627		TD	sensitivity	0.450	0.733	0.770	0.780	0.682	0.490
		specificity	0.911	0.922	0.905	0.930		LK	specificity	0.906	0.910	0.937	0.922	0.971	0.933
	OT.	sensitivity	0.823	0.913	0.873	0.763		CI	sensitivity	0.503	0.707	0.887	0.787	0.767	0.647
	SL	specificity	0.929	0.971	0.966	0.924		5L	specificity	0.917	0.939	0.971	0.965	0.941	0.927
	D.C.	sensitivity	0.803	0.916	0.874	0.756		DE	sensitivity	0.493	0.687	0.880	0.797	0.760	0.613
7	RF	specificity	0.931	0.966	0.961	0.924	7	RF	specificity	0.917	0.939	0.969	0.957	0.929	0.935
	ODM	sensitivity	0.822	0.928	0.870	0.742	7	ODM	sensitivity	0.510	0.720	0.897	0.790	0.770	0.617
	GBM	specificity	0.926	0.963	0.967	0.930		GBM	specificity	0.918	0.936	0.967	0.962	0.940	0.937
	T D	sensitivity	0.706	0.789	0.837	0.634		I D	sensitivity	0.457	0.667	0.787	0.780	0.697	0.483
	LK	specificity	0.921	0.919	0.897	0.918		LK	specificity	0.906	0.927	0.929	0.926	0.953	0.933

Table	A 4	continue	d.



Figure A 1 An example of 12 hours of diving from a 40kg juvenile Australian fur seal. Panels show VeDBA, raw acceleration of the x, y and z axis, location (underwater, surface or land), behaviour state (travelling, resting, grooming, foraging) and depth