INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600

PHENOTYPE AND MATING SUCCESS OF MALE HARBOUR SEALS, *PHOCA VITULINA*, AT SABLE ISLAND, NOVA SCOTIA

by

David W. Coltman

Submitted in partial fulfillment of the requirements

for the degree of Doctor of Philosophy

at

Dalhousie University

Halifax, Nova Scotia

December 16, 1996

DALHOUSIE UNIVERSITY

FACULTY OF GRADUATE STUDIES

The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled "Phenotype and Mating Success of Male Harbour Seals (*Phoca vitulina*) at Sable Island, Nova Scotia"

by David William Coltman

in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Dated: December 9, 1996

[redacted]

External Examiner

Research Supervisor

Examining Committee

DALHOUSIE UNIVERSITY

DATE: December 18, 199

AUTHOR: David William Coltman

TITLE: Phenotype and mating success of male harbour seals, PHOCA VITULINA, AT Sable Island, Nova Scotia.

DEPARTMENT OR SCHOOL: Biology

DEGREE: Ph.D. CONVOCATION: May YEAR: 1997

Permission is herewith granted to Dalhousie University to circulate and to have copied for non-commercial purposes, at its discretion, the above title upon the request of individuals or institutions.

Signature of Author

To my mom:

For your unconditional love, support and understanding,

you have always been there for me.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Reproductive behaviour and mating success of male harbour seals (*Phoca vitulina*) were studied at Sable Island, Nova Scotia, between 1992 and 1995. Individually marked males were fitted with time-depth recorders to monitor their activity at sea. Males were also given deuterium labelled water to estimate body composition and energy expenditure during the breeding season. These studies showed reproduction to be as energetically costly for male harbour seals as it is for males of other species. Due to small body size, most male harbour seals cannot energetically afford the extended fast which is characteristic of other breeding pinnipeds, hence they forage during deep diving bouts until females are likely to become receptive. All males changed their diving behaviour during the mating period such that repeated bouts of shallow diving activity, which reflect reproductive behaviour, predominated.

Mating success was estimated using microsatellite markers to analyze paternity genetically. The paternity analysis of two cohorts of pups indicated that harbour seals are polygynous, as the most successful male harbour seals may sire as many as 6 pups in a season, but the variance in mating success among males was low, with most males likely to have sired one or no pups. This is consistent with the limited ability of males to monopolize females in the aquatic mating environment, and with the dispersal of females at sea near Sable Island. Females may mate with males from any stretch of the island.

Male mating success could not be reliably predicted from any single phenotypic characteristic, however, multivariate analysis identified suites of characteristics which are associated with varying degrees of reproductive success. Relatively large males which hauled out alone and bore wounds from fighting were reproductively unsuccessful, and likely represent individuals who have been defeated in intrasexual competition. The group of males with the highest rate of mating success tended to have the greatest energy stores at the beginning of the mating season, and they started to compete for mates earlier and at greater energetic expense than other males.

ABBREVIATIONS

ΔLOD	-	Difference in logarithm of odds
ΔTBE	-	Daily change in total body energy
ATP	-	Adenosine triphosphate
ATV	-	All-terrain vehicle
BW	-	Body water pool
CV	-	Coefficient of variation
D_2O	-	Deuterium oxide
DNA	-	Deoxyribonucleic acid
f(ex)	-	Frequency of exclusion
FEI	-	Food energy intake
FI	-	Food intake
FMR	-	Field metabolic rate
FWI	-	Food water intake
HWE	-	Hardy-Weinberg equilibrium
k	-	Fractional rate of water turnover
LOD	-	Logarithm of odds
MS_{max}	-	Maximum estimate of mating success
MS_{ML}	-	Most-likely mating success
MWP	-	Metabolic water production
n	-	Sample size
P(ex)	-	Probability of exclusion
P(id)	-	Probability of identity
P_{MS}	-	Probability of mating success
PCR	-	Polymerase chain reaction
TBE	-	Total body energy
TBF	-	Total body fat
TBP	-	Total body protein
TBW	-	Total body water
TDR	-	Time-depth recorder
TEE	-	Total energy expended
TWF	-	Total water flux

ACKNOWLEDGEMENTS

I find it difficult to believe that my four and a half years in Halifax are over. It seems like yesterday that I was laying awake in bed wondering how it could all be done. This project would not have been possible without the advice, assistance and support of many people.

I am most grateful to my supervisor and mentor, Don Bowen. Thank you for entrusting me with this project, and for giving me the inspiration, opportunity and philosophical training to become a scientist. Thank you also for always finding the time to talk to me when I needed your feedback and sense of perspective. As well, I will not forget that I owe Sara Iverson "big time". More than anything Sara, you made science and fieldwork on Sable Island fun, and taught me the meaning of teamwork. From the start, both of you treated me like family when I was a long way from home, and I will be forever grateful.

Many others assisted me on Sable Island. Daryl Boness was always willing to answer questions, provide insight and share a laugh. Most of all Daryl, thanks for always being willing to listen. Special thanks to Jim McMillan for 4 years of friendship, good humour and seal catchin' fun. Thanks also for providing an even keel during the occasional periods of emotional turbulence. To Monica "momma bear" Muelbert, I missed you dearly in the last year of my Ph.D., especially on Sable Island. It just wasn't the same when you were gone.

Other people provided useful hands in the field as well- thanks to past and present teamates Brian Beck, Keith Jensen, Penny Kirsch, Sara Ellis and

Sable Island denizens including the OIC- Gerry Forbes.

I am indebted to many others at Dalhousie University. On the sixth floor, Dr. Jon Wright gave this ecologist the opportunity to become a geneticist. Thank you Jon, for giving me a chance, and for teaching me about practical aspects of a career in science. I am also grateful to Eileen Denovan-Wright for her advice and moral support. Dr. Ian McLaren was always receptive and keen to provide insight. Labmates Andrew, Joyce, Jenny, Marek, Louis, Lorraine, Pat and especially Mary Dillon and Kenny Richard, were sources of information and support. In the MGPL, Mick O'Connell and Stuart McConnell provided advice, and I relied on Doug Cook for more practical genetic wisdom than any other single person. I am also grateful to Simon Goodman and Neil Gemmell (Cambridge) for sharing primers and Tristan Marshall (Edinburgh) for PATSIM.

My friends, where do I start? Merilee Temple, you'll always have a place in my heart, and I'll never forget the good times that we had. To my cronies Steve MacIntosh, Brian Dixon, Brent Sutherland, Vagelli Panopalis, John Shears, Derek Coronado, Rick White, June Trillo and my cat Bismarck- thanks for the memories, beverages, and other amusements. To Steve K., Kate and Helen, I'll always love you dearly. You provided me with a family in Halifax, for that, I'm eternally grateful. Thanks for the fajitas and Scrabble© sessions too.

Finally, to my family in Toronto, my brother Chris and mom, thanks for your love and moral support. Mom, I owe everything to you for your encouragement.

This work was supported by funds from NSERC, DFO and the Lett fund.

CHAPTER I

GENERAL INTRODUCTION

Studies of the extent and cause of the variance in reproductive success among individuals in natural populations are important for understanding processes of selection, adaptation and population demography (Clutton-Brock 1988). Modern approaches to the study of mating systems and reproductive success are firmly grounded in Darwin's theories of natural and sexual selection (Darwin 1858; Darwin 1871); that is, mating systems are most usefully viewed as a product of the reproductive strategies of individuals rather than as a characteristic of a population or a species (Emlen and Oring 1977; Orians 1969; Clutton-Brock 1989). A reproductive strategy basically describes how an individual allocates reproductive effort into parental care and/or mating to achieve reproductive success, and ultimately in order to maximise their genetic contribution to future generations.

The evolution of lactation in mammals has left females with the principal responsibility for parental care, therefore most male mammals may best maximize their reproductive success by investing their reproductive effort towards mating, whereas female reproductive success can best be improved by securing adequate food resources and a safe site for parturition and lactation (Trivers 1972). Without the competing demands of parental care, the upper limits of male reproductive success are constrained only by the number of females a

male can successfully inseminate, hence the potential for polygyny (i.e., a large variance in male reproductive success) is generally high among mammals (Emlen and Oring 1977) and most mammals are polygynous (Kleiman 1977). However, ecological constraints associated with the spatial and temporal distribution of females, coupled with their economic defensibility, will largely determine the extent to which polygyny will occur (Emlen and Oring 1977) and thus play a pivotal role in shaping male reproductive strategies. When receptive females are clustered in space and time, some males may be more able to monopolize access to mates by defending resources which are important for female reproductive success (resource defence polygyny), or by controlling groups of potentially receptive females (female defence polygyny). When females or their resources cannot be controlled by males, or they are widely or unpredictably distributed, males may either search for individual receptive females and court them sequentially (serial monogamy or sequential polygyny) or attract females by advertising on a mating territory or home range (Clutton-Brock 1989). Tightly clustered or overlapping male mating territories are also known as leks (Bradbury and Gibson 1983) and are rare among mammals (Clutton-Brock 1989; Davies 1991).

Pinnipeds are morphologically adapted to life at sea, but must return to shore to give birth to their pups, making them vulnerable to terrestrial predators during the breeding season (Bartholomew 1970; Stirling 1983). Females are therefore limited in their choice of safe breeding sites, and where they breed on isolated islands or protected beaches they can be very tightly spatially clustered,

which facilitates polygyny (Bartholomew 1970). Other species breed on seasonally forming pack ice, where there is an almost unlimited choice of pupping sites and females may be more widely spaced, which reduces the number of females that a single male can simultaneously attend (Stirling 1983). Most pinnipeds are also seasonal breeders, which means that oestrus is usually synchronized to the extent that receptive females are predictably clustered in time during the breeding season, thereby providing the opportunity for a small number of males to dominate matings (Stirling 1983). However, Boness (1991) also noted that the degree of synchrony may also limit male mating success among pinnipeds if most females become receptive at the same time (highly synchronous), or over a duration of time which is longer than males can energetically afford to compete for females (asynchronous) unless males return to sea to feed.

When mating occurs on land, successful males may achieve maximum mating success through controlling access to females by defending groups of females on land such as grey seals, *Halichoerus grypus*, (Boness and James 1979) and northern elephant seals, *Mirounga angustistrostris*, (Le Boeuf 1974). Males in other species, such as the northern fur seal, *Callorhinus ursinus*, (Bartholomew and Hoel 1953) may defend the boundaries of territories within the breeding colony. Male body size is generally thought to be an important phenotypic characteristic in these species. In southern elephant seals, *Mirounga leonina*, body size confers a fighting advantage which enables large males to maintain high dominance status (McCann 1981; Modig 1996). In other species

large size enables males to prolong their period of tenure in the breeding colony since males fast while onshore throughout the breeding season and large body size favours fasting endurance due to the scaling of body size and metabolism (Lindstedt and Boyce 1985). Consequently, pronounced size dimorphism is generally believed to have evolved in terrestrially breeding pinnipeds in response to intense sexual selection on male body size (Bartholomew 1970).

Considerably less is known about reproductive strategies and the mating systems of aquatically mating pinnipeds. Since most phocid seals mate in the water, aquatic mating is a dominant feature in the mating systems of about one-half of the extant pinniped species (Stirling 1983; Le Boeuf 1991; Boness 1991). This lack of information is a direct result of the inherent difficulty in studying behaviours which occur at sea, and has been identified as a major gap in our general understanding of pinniped mating systems (Le Boeuf 1991; Boness 1991; Boness **et al.** 1993). It was initially thought that polygyny ought to be reduced among aquatically mating pinnipeds, since the three dimensional nature of the mating environment and the considerable mobility of seals in the water make it difficult for a male to limit the access of competitors to females, the movements of females, or to predict female locations at sea (Bartholomew 1970). On another level, however, the variance in male mating success may be high given the potential for females to exercise choice in mating, and aquatic displays appear to be an important component of male reproductive behaviour in several aquatically mating species (Le Boeuf 1991).

Size dimorphism is generally less pronounced or absent in aquatically mating species, perhaps because agility may be more important than size for a male's competitive ability in a weightless environment (Le Boeuf 1991). Secondly, since mating and foraging both occur in the aquatic environment, fasting endurance may be less important to the reproductive success of aquatically mating males since they may be able to forage opportunistically or effectively budget their time between feeding and competing for mates during the breeding season. Thirdly, if the variance in male mating success is low the intensity of sexual selection on male size may be reduced.

The harbour seal, *Phoca vitulina concolour*, is a slightly dimorphic, relatively small-bodied pinniped (McLaren 1993) which breeds on isolated islands or beaches along the eastern coast of North America (Boulva and McLaren 1979). Harbour seals mate aquatically, but relatively little is known about male reproduction since they mate at sea and they are difficult to approach and capture in most populations, making longitudinal studies of more than a few individuals difficult (Reilly and Fedak 1991). Each year, females come ashore, give birth and nurse their pups during a relatively short breeding season (4-6 weeks), and probably mate sometime in late lactation (Boulva and McLaren 1979; Thompson 1988). Receptive females are therefore predictably clustered geographically and temporally such that polygyny is possible. At the same time, mating occurs at sea and size dimorphism is weak, which predicts that polygyny may be reduced (Stirling 1983). Data on mating success is scant, however.

Recent advances in molecular genetic techniques now enable more accurate estimates of mating success than given by observed copulations, and the advent of time-depth recorder technology and methods using stable isotope dilution provides the opportunity to gain a better understanding of male energetics and behaviour at sea in species which mate aquatically. This study is an analysis of the reproductive behaviour and energetics of aquatically-mating male harbour seals in relation to mating success determined by a molecular genetic analysis of paternity.

In Chapter II, I describe patterns of diving behaviour, mass loss and the influence of body size on the aquatic activity of individual male harbour seals. The collection of longitudinal diving data using time-depth recorders and mass loss over the breeding season enabled me to describe cycles of aquatic behaviour associated with foraging and reproduction in relation to the availability of oestrus females in the colony. Differences in the aquatic activity patterns of relatively small and large males are used to infer the influence of body size on reproductive behaviour, and ultimately to make predictions about the influence of body size on reproductive success.

Chapter III describes the energetic costs of reproduction for individual males using stable isotope dilution. These experiments enabled me to examine energy expenditure from initial body stores, and the energetic input gained from foraging during the breeding season in relation to body size and behaviour at sea. Energy is a currency of reproductive effort, hence I hypothesized that body size and the amount of stored energy males possess at the beginning of the

season play a role in determining how males partition their activity between foraging and reproduction during the breeding season. The energetic costs of aquatic mating to male harbour seals are also compared to the costs of mating to males of polygynous, terrestrially mating pinniped species.

In Chapter IV, I describe the development and use of microsatellite genetic markers to determine paternity in two cohorts of pups born on Sable Island, and thereby estimate the variance in mating success among male harbour seals. I describe the variance in male mating success and compare the implied level of polygyny between aquatically mating harbour seals and estimates from terrestrially mating pinnipeds. I use this analysis to test the hypothesis that polygyny is reduced in this aquatically mating pinniped.

Finally, in Chapter V I describe the phenotypic characteristics which are associated with male mating success. Using a combination of observable behaviours, body size, mass change and paternity data I classify individuals based on their overall patterns of behaviour and mating success. Comparisons of energetic variables and aquatic behaviour in relation to the availability of oestrus females between groups of males which differ qualitatively in their overall behaviour patterns and mating success identify the phenotypic characteristics which may predict reproductive success in an aquatically mating pinniped.

CHAPTER II

CHANGES IN THE DIVING BEHAVIOUR OF ADULT MALE HARBOUR SEALS DURING THE BREEDING SEASON.

INTRODUCTION

Observational studies of the reproductive behaviour of male pinnipeds usually focus on interactions between adult males and females on shore. Among species in which males haul out throughout the breeding season and mate terrestrially, it is possible to investigate links between body size, reproductive energetic effort, activity patterns and reproductive success through direct observation and serial weighings of marked individuals (Boness 1984; Anderson and Fedak 1985; Deutsch **et al.** 1990; Boyd and Duck 1991; Tinker **et al.** 1995).

A large number of pinniped species mate aquatically. Fifteen of the 18 phocid seals mate exclusively in the water and aquatic copulation occurs frequently in several otariid (i.e., fur seals and sea lions) species (Boness **et al.** 1993). The mating systems of aquatically copulating pinnipeds have not been studied extensively due to the inherent difficulties in observing reproductive behaviour which occurs at sea. This has been identified as a major gap in our overall understanding of the reproductive strategies of male pinnipeds (Le Boeuf 1991; Boness 1991; Boness **et al.** 1993).

The harbour seal is perhaps the most widely-studied aquatically mating pinniped. However, most studies of male reproductive behaviour have been

limited to observations at the haul-out site (Sullivan 1981; 1982; Davis and Renouf 1987; Godsell 1988; Thompson 1988; Walker and Bowen 1993ab). Although some authors (Godsell 1988) believe that behavioural interactions between males and females on shore may be unrelated to mating strategies of males at sea, such studies have provided some important insights into harbour seal mating behaviour. For example, the timing of the appearance of lacerations on the neck and hindflippers of males resulting from intrasexual competition approximately coincides with the presence of increasing numbers of oestrus females (Davis and Renouf 1987; Thompson 1988; Walker and Bowen 1993b), suggesting that males intensify competition for mates in the water towards the latter part of the breeding season. Observations of increased rates of mass loss and the absence of chylomicrons (indicative of recent feeding) in male serum during late lactation (Walker and Bowen 1993a) also suggest that harbour seal males change from foraging activity to competition for mates as more oestrus females become available.

Using VHF transmitters, Thompson **et al.** (1989) observed that males make shorter diving trips during the breeding season, suggesting a reduction in foraging activity near the time of mating. More recently, through VHF telemetry and acoustic recordings, Van Parijs **et al.** (1996) found that male harbour seals reduce their home range size and make shorter dives which are associated with underwater vocal displays during the mating period of the breeding season. Underwater vocalisations associated with display dives appear to be individual-

specific, suggesting that they are used in male-male competition, as reproductive advertisement to attract females in the water, or both (Hanggi and Schusterman 1994). In the Moray firth, Van Parijs **et al.** (1996) also found that males tended to perform these displays at locations where they are likely to intercept females during the breeding season; either on foraging grounds, around haul-out sites, or on transit routes between these two areas.

At Sable Island, evidence from time-depth recorders (TDRs) indicated that female harbour seals spent more time in the water as they resume foraging trips during mid-lactation, however, females returned to the beach daily to nurse their pups (Boness **et al.** 1994). These findings suggest that male harbour seals may maximise their encounter rates with potentially oestrus females by discontinuing foraging activity and attempting to intercept or attract females in nearshore waters as females depart from or return to the breeding colony in late lactation and following weaning. I hypothesised that male diving patterns should reflect a transition from offshore, presumably deep-diving, foraging activity to shallow dives associated with inter-male aggression and mating later in the breeding season. Thus, I also predicted an inverse relationship between individual rates of mass loss and deep diving activity, and conversely, a positive correlation between shallow diving and mass loss throughout the breeding season. Finally, since larger seals start the breeding season with larger absolute energy stores, they should need to spend less time feeding; I predicted that

large seals would have different activity patterns than smaller seals, reflecting less effort devoted to foraging.

METHODS

The study was conducted during the breeding seasons (mid-May to the end of June) of 1992, 1993 and 1994 on the north beach of Sable Island (43°55'N; 60°00'W), a partially vegetated sandbar 160 km east of Halifax, Nova Scotia. Between 300 and 500 harbour seal females gave birth on Sable Island during each year of the study.

Male harbour seals were captured, marked, and weighed as described by Walker and Bowen (1993ab) near the beginning of each breeding season (range 20 May - 30 May). Once secured in nets, seals were sedated to minimise stress during handling, and a dorsal standard length measurement (McLaren 1993) was taken. In each season, twelve adult seals with initial mass greater than 85kg were fitted with a Mk3+ TDR (Wildlife Computers, Woodinville WA) containing 256K of memory as described in Boness **et al.** (1994). TDRs weighed approximately 300g, representing less than 0.4% of initial body weight (range 0.22 - 0.35 %). Males were recaptured near the end of the breeding season (range 15 June - 1 July) for TDR removal and re-weighing.

Analysis of Dive Records

TDRs were programmed to record depth every 10 seconds. Data from each TDR were processed with software provided by the manufacturer. Data files were initially examined graphically (Strip Chart program) to provide a visual

representation of the diving pattern of each male (Figure 2.1) and then corrected for changes in the calibration of the TDR's pressure transducer (Zero Offset Correction program) over the data collection period. Corrected files were analyzed (Dive Analysis program) to provide estimates of individual dive parameters for each record. I used the default definitions found in the Dive Analysis program to describe the following parameters:

dive duration - the duration between the time of the surface readings immediately preceding and following the dive, minus one sampling interval.

dive depth - the maximum depth reading that was taken during the dive.

bottom time - the time between the first and last depth reading equal to or greater than 15% of the dive depth.

average rate of ascent and descent - the average rate of descent was calculated from the start point of each dive to the beginning of bottom time. The average rate of ascent was calculated from the end of bottom time to the end point of the dive.

time hauled-out - the total time seals spent dry.

It is only feasible to correct for transducer drift in blocks of dives, therefore dives to 4m maximum depth or less were not analyzed since

Figure 2.1. Illustrations of male harbour seal diving behaviour produced by the Strip Chart program (Wildlife Computers). Figure 2.1A shows the diving pattern of male S6933 (1993) during the entire deployment interval (24 days). Figures 2.1B and C illustrate an individual deep (B) and shallow (C) diving bout, while Figures 2.1D and E depict the typical shapes of dives occurring within deep (D) and shallow (E) diving bouts.

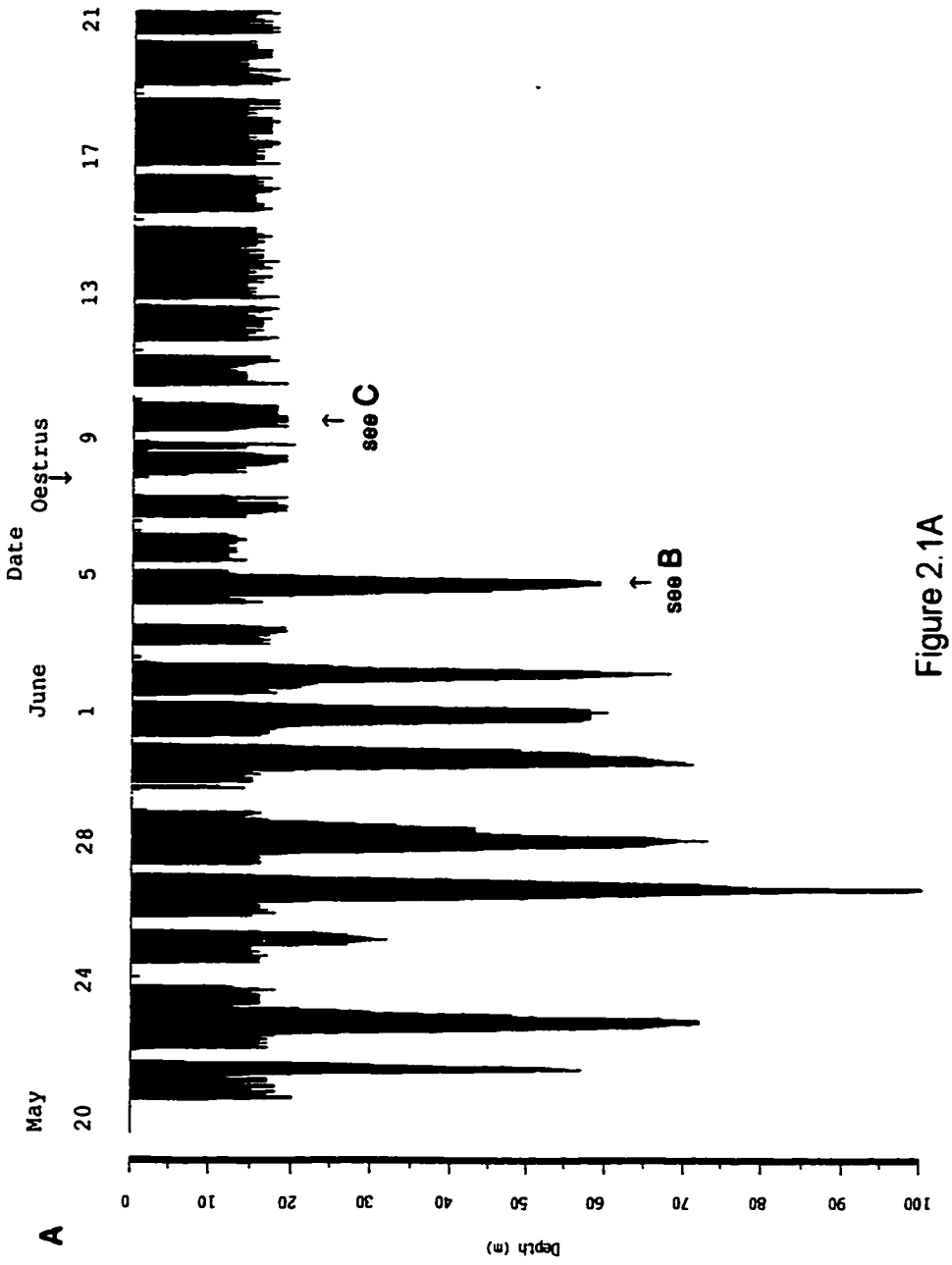

A
Depth (m)
0
10
20
30
40
50
60
70
80
90
100
May
20
24
28
June
1
5
Date
Oestrus
9
13
17
21
see B
see C

Figure 2.1A

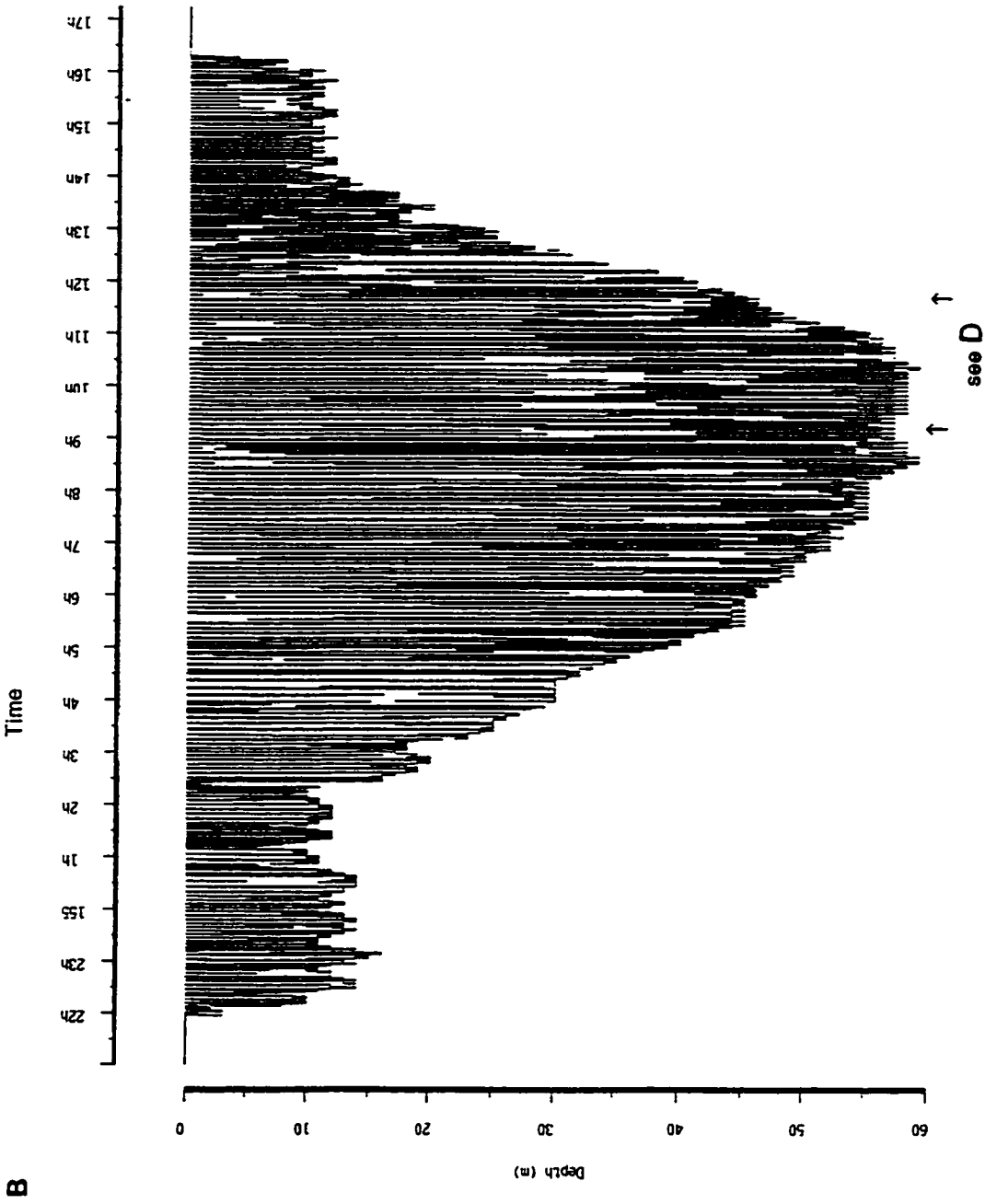


Figure 2.1B

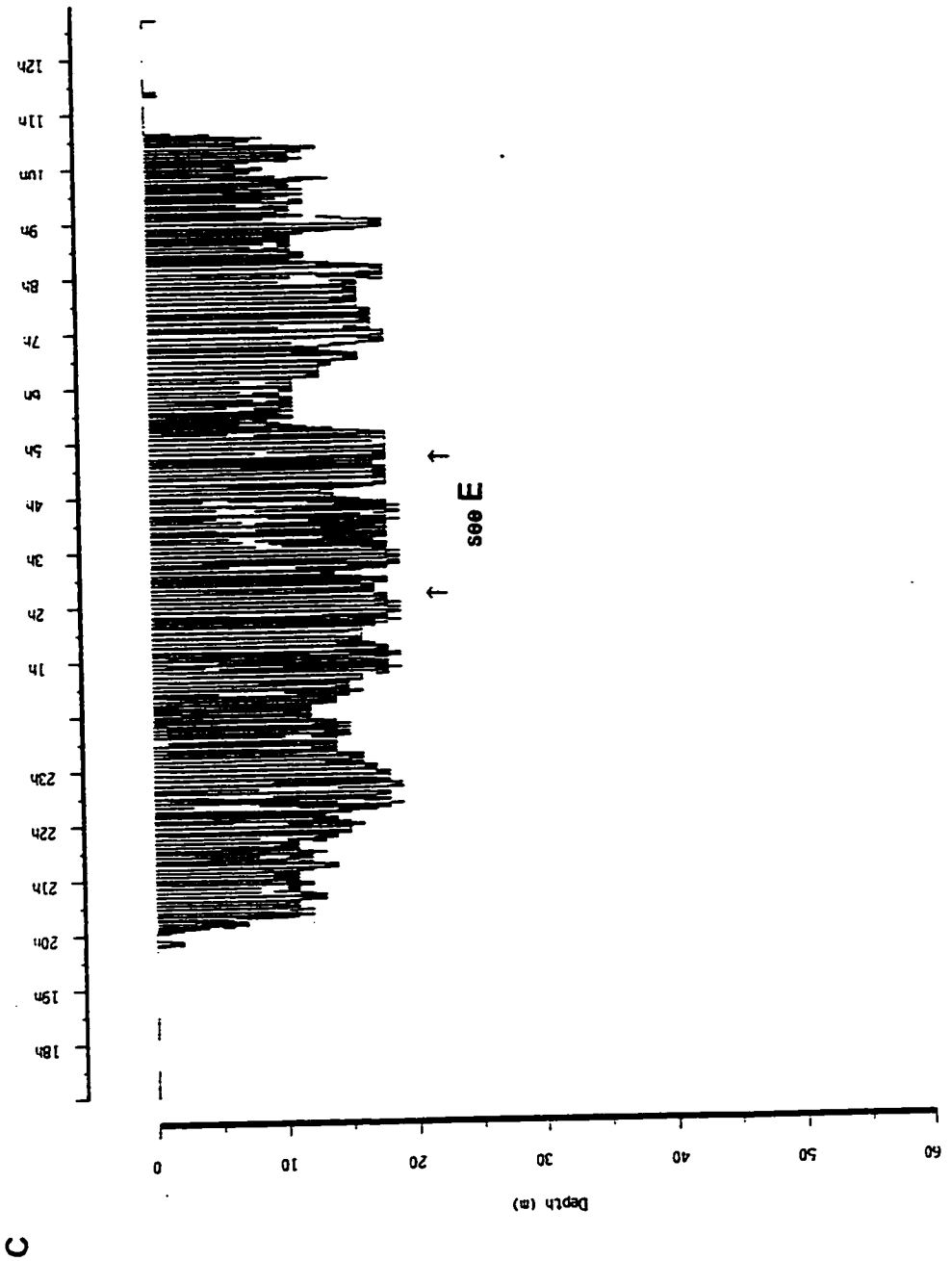

C
Depth (m)
0
10
20
30
40
50
60
see E
1h
2h
3h
4h
5h
6h
7h
8h
9h
10h
11h
12h
18h
19h
20h
21h
22h
23h

Figure 2.1C

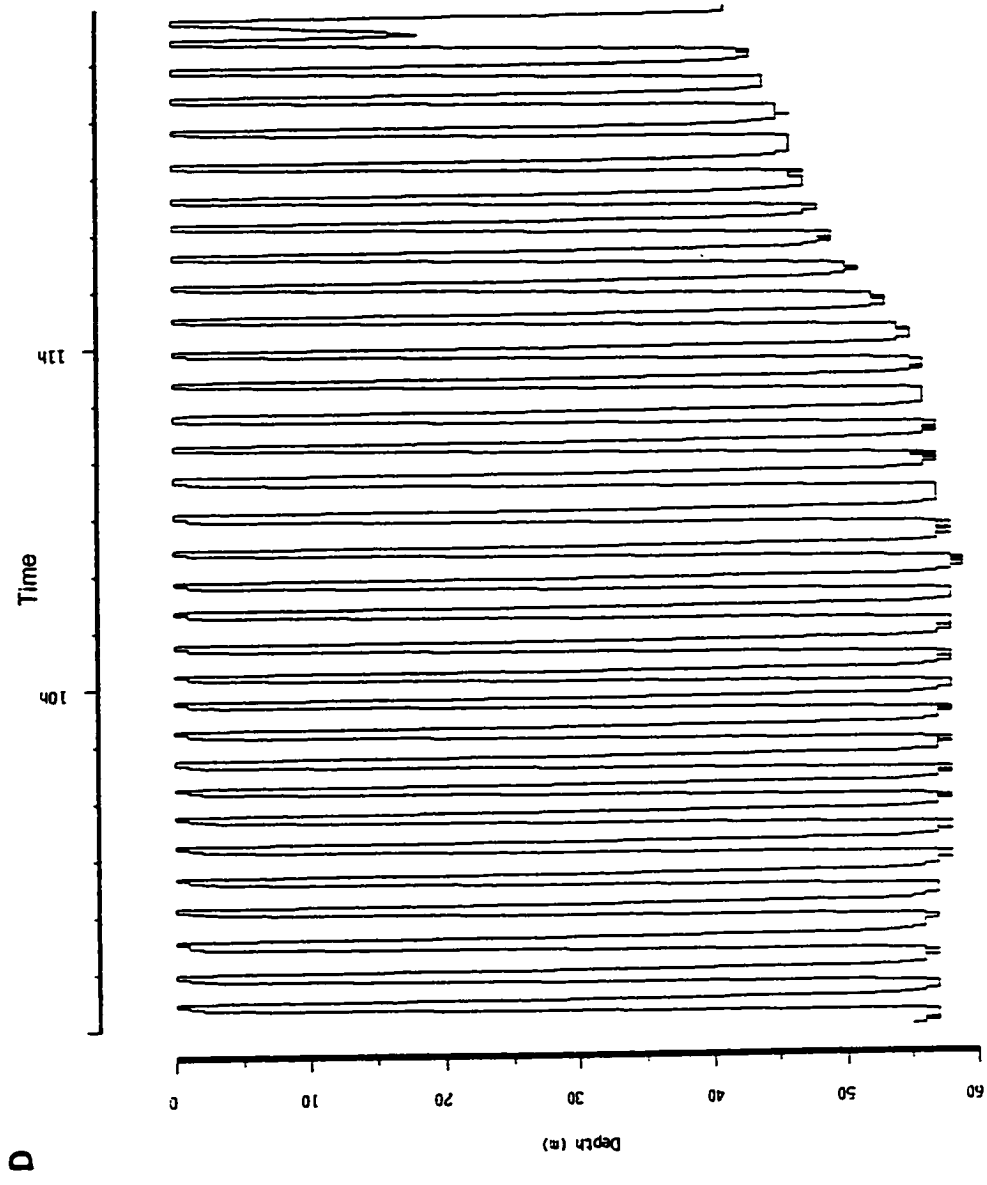


Figure 2.1D

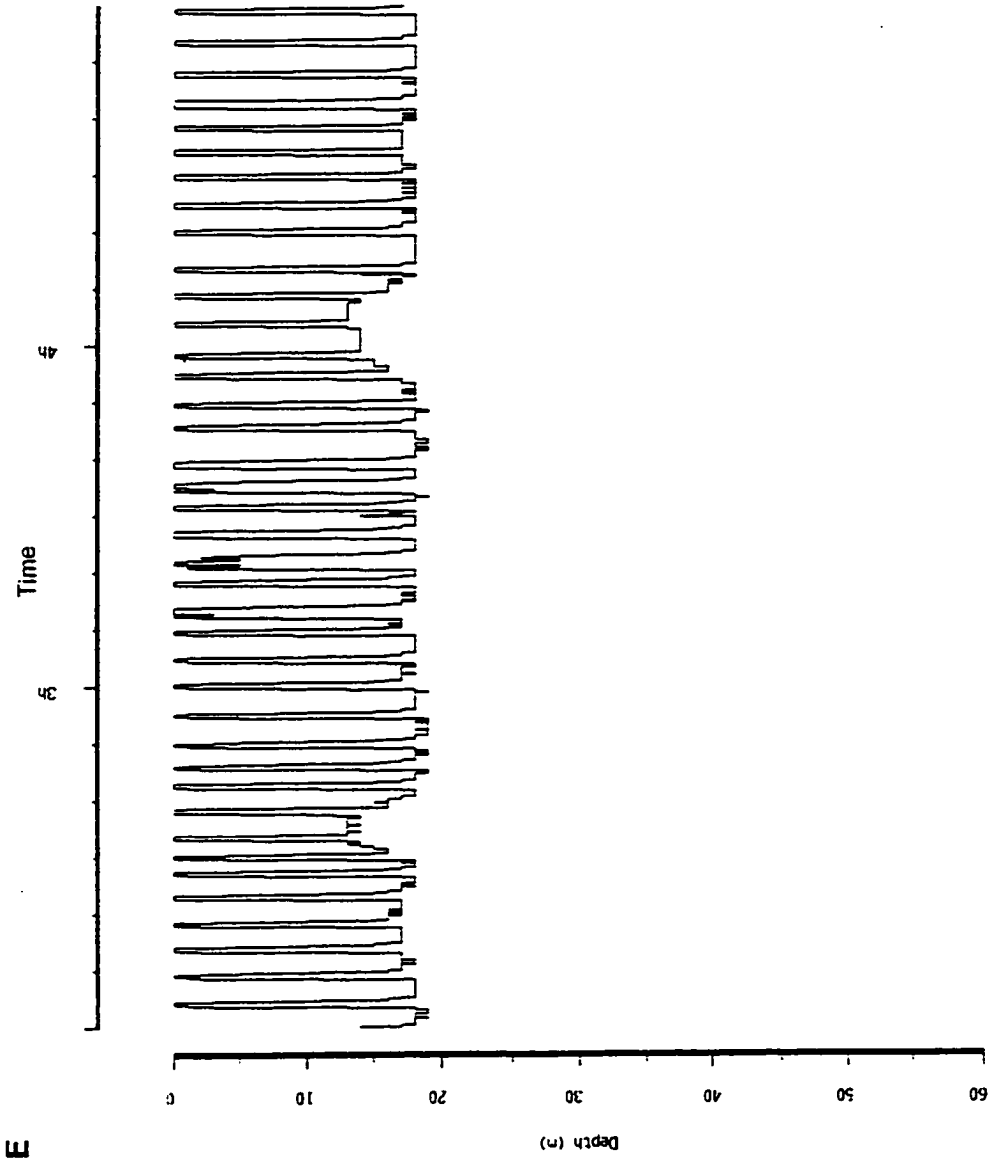


Figure 2.1E

instrument noise causing drift slightly greater than 2m (twice the depth resolution of the TDR) might have been recorded as shallow dives.

Based on an inspection of the overall dive patterns (Figure 2.1A), I defined 2 major classes of dives. Individual dives to depths ≤ 20m were considered 'shallow', and dives to maximum depths of > 20m were considered 'deep'. Bathymetric maps of waters surrounding Sable Island indicate that dives to maximum depths > 20m must occur at least 2 nautical miles offshore, whereas, if seals are diving to the bottom at depths ≤ 20m they are relatively close to the breeding colony.

Since diving tended to occur in clusters, or bouts, of dives either ≤ 20m or considerably greater (Figure 2.1B and C), I also defined 2 types of bouts to characterize the temporal aspects of diving patterns. The operational definition of the onset of a bout was the occurrence of 5 consecutive dives of 5m or greater followed by at least 5 deeper dives. Dive bouts were considered to end when an animal remained at the surface for at least 1h or returned to land. Visual inspection of graphic representations of some individual bouts suggested that there are two major types of bouts. 'Shallow' bouts tended to start with a small number of dives with increasing depth, then an extended period of repeated diving to a median depth ≤ 20m (Figure 2.1C). 'Deep' bouts consisted of clusters of dives with a median depth > 20m, commonly reaching maximum depths > 50m (Figure 2.1B).

Data Analysis

To examine the effect of initial body mass on male diving behaviour and mass change, I divided the sample into two groups based on the median value (108.0 kg) of initial mass measurements. Males with initial mass ≤ 108 kg were considered small, and heavier males were classified as large. To investigate the extent to which the diving behaviour of large and small males differed before and after oestrus females were likely to be present in the colony, I divided the breeding season into a pre-mating and mating period based on similar criteria described previously in Walker and Bowen (1993ab). Mating is thought to occur near weaning (Boulva and McLaren 1979; Thompson 1988). On Sable Island, the first pups were born on 15 May, 13 May, and 14 May in 1992, 1993, and 1994, respectively. Assuming a mean lactation duration of 24 days (Muelbert and Bowen 1993), oestrus females would have become increasingly numerous after about 8 June in each year (the median expected date of the appearance of the first weaned pup). I used this date to divide the breeding season into the 'pre-mating' and 'mating' periods.

Statistical analyses were usually performed using Minitab Release 10 for Windows. Prior to statistical analyses, all data were checked for normality, transformed if necessary, and in the case of multivariate analyses, converted to a mean of zero and standard deviation of 1. Unless otherwise stated, the probability level accepted for statistical significance was set at $\alpha = 0.05$ and

standard errors are given as the measure of variance about the mean. Regression analyses for changes in dive parameters over time were not performed due to the lack of independence of measures for consecutive days and the variable length of TDR deployment among individuals. Repeated measures analysis of variance was used to compare the activity of large and small males between the pre-mating and mating periods using SPSS.

RESULTS

I failed to recover data from 5 TDRs due to loss of the instrument at sea (n = 1), battery failure (n = 3) and the infusion of seawater into the unit (n = 1). Therefore, data from 31 TDRs were used in this analysis (see Table 2.1). Deployment duration differed significantly between years (one-way ANOVA; df = 2, 28; $F = 75.17$; $P < 0.001$), with a significantly longer mean deployment period in each successive year. Mean body mass and dive characteristics (Table 2.1), corrected for the number of days in the deployment period, did not differ significantly between years (MANOVA; df = 14, 44; $F = 1.26$; $P = 0.272$; variables tested were: average initial mass, length, rate of mass change (kg/day), percent of dives >20m, dive effort, dives per day and bouts per day), therefore data between years were pooled for all subsequent analyses.

Characteristics of Individual Dives

The average characteristics of shallow and deep dives for the 31 males are shown in Table 2.2. Although the number of shallow and deep dives per day was variable among males, with coefficients of variation (CV) of 71.6% and 59.5%, respectively, records contained significantly more shallow dives than deep (two-tailed paired t-test; n = 31; $t = 3.79$; $P < 0.001$). Coefficients of variation were uniformly higher for shallow dive characteristics than for deep dive characteristics. Deep dives tended to be rather uniform both within records

Table 2.1. Body size and diving characteristics of TDR-fitted adult male harbour seals (n=31).

ID	Year	Initial mass (kg)	Final mass (kg)	Record duration (days)	Number of dives	Number of bouts	Time hauled-out (min h^{-1})	Dive effort (min h^{-1})	Percent of dives >20m deep
S6853	1992	115.5	101.0	22	1859	11	9.2	16.04	68.20
S6854	1992	111.0	101.0	23	6603	11	5.2	41.10	45.11
S6587	1992	117.0	100.0	24	4951	31	10.5	27.90	26.68
S6856	1992	115.5	102.0	22	2954	26	19.9	13.38	3.14
S6857	1992	102.0	91.5	23	4753	18	19.3	29.14	57.94
W116	1992	114.5	107.0	21	2061	25	12.6	13.26	21.39
S6860	1992	111.5	98.5	22	4182	20	11.7	28.62	31.90
S6861	1992	107.0	106.5	22	3636	13	10.2	31.41	81.57
W119	1992	120.5	113.5	22	3220	16	19.7	24.37	51.35
S6863	1992	106.5	99.5	22	1535	16	na	11.85	48.73
S6866	1992	108.0	99.0	22	6484	10	5.8	37.99	19.91
S6864	1992	108.0	104.0	21	1804	8	na	13.60	61.14
S6921	1993	103.0	100.0	25	5182	13	5.3	33.06	65.48
3032	1993	98.5	90.5	24	2615	26	16.7	15.21	58.16
S6920	1993	111.0	90.0	25	4246	24	14.2	19.12	9.80
S6925	1993	131.5	111.5	30	7695	22	11.0	32.27	18.90
S6927	1993	96.5	85.5	24	6429	29	21.2	23.05	11.59
S6928	1993	112.5	95.0	30	5701	30	19.4	31.29	46.17
J9162	1993	99.5	90.5	26	2762	24	14.2	16.14	57.13
S6933	1993	88.5	85.0	25	6167	24	5.8	34.99	16.96
S6945	1993	127.0	114.5	23	7234	16	1.3	38.38	6.66
S6951	1993	85.0	74.5	29	4683	18	28.7	24.49	62.37
S6990	1994	96.0	90.0	38	4800	17	24.1	21.44	65.02
S6862	1994	110.5	99.5	34	5966	25	10.4	28.01	41.67
S6998	1994	105.5	91.5	34	5516	24	6.3	29.63	51.27
L11307	1994	98.0	92.8	32	5512	32	11.8	29.63	27.34
S6577	1994	111.5	88.5	35	9647	35	9.8	35.49	15.45
L11203	1994	107.0	88.3	31	4840	31	24.1	28.15	40.50
S6986	1994	103.5	88.3	30	2060	30	35.4	11.86	71.17
S6958	1994	111.5	87	32	11977	32	2.4	34.89	5.86
L11222	1994	116.5	83	32	10029	32	1.6	34.69	1.29

Notes:
na- data not available due to instrument failure.

Table 2.2. Characteristics of shallow and deep dives made by adult male harbour seals during the breeding season near Sable Island, Nova Scotia (n = 31).

Parameter	Shallow (<20m) dives			Deep (>20m) dives		
	Mean	Range	CV (%)	Mean	Range	CV (%)
Number of dives (day^{-1})	127 ± 16	20 to 353	71.6	61 ± 7	4 to 136	59.5
Maximum depth (minutes)	10.5 ± 0.5	6.1 to 17.0	27.6	38.8 ± 2.2	25.9 to 78.4	30.9
Duration (minutes)	3.0 ± 0.1	1.8 to 4.4	18.2	4.6 ± 0.1	3.7 to 5.6	11.1
Time at bottom (minutes)	1.6 ± 0.1	0.8 to 2.9	71.6	3.1 ± 0.1	2.4 to 4.0	12.7
Ascent rate (ms^{-1})	0.42 ± 0.02	0.24 to 0.62	22.1	0.90 ± 0.02	0.61 to 1.19	14.9
Descent rate (ms^{-1})	0.40 ± 0.02	0.25 to 0.65	24.4	0.81 ± 0.02	0.60 to 0.98	12.8
Time since last repeated dive (minutes)	1.31± 0.14	0.55 to 4.00	58.1	0.98 ± 0.02	0.75 to 1.34	12.6

and between seals, with a symmetrical, "flat-bottomed" shape and similar rates of ascent and descent (see Figure 2.1D and E). Shallow dives were more variable in shape, particularly with respect to the amount of time spent at the bottom (CV=71.6%). While many shallow dives appeared to be "flat-bottomed", there were also many "V" shaped dives with little time spent at the maximum depth (see Figure 2.1E). Shallow dives were significantly shorter, had less bottom time, and slower average rates of ascent and descent than deep dives (MANOVA of standardized parameters; $df = 4, 57$; $F = 120.31$; $P < 0.001$).

The average characteristics of all dives clearly changed over the breeding season in relation to the availability of oestrus females (Figure 2.2A-F). In the mating period, dives generally became shallower (the average dive depth declined from 20-25m to 10-15m) and shorter (4.0-4.5 minutes vs. 2.0-3.0 minutes), with less time spent at the bottom (2.5-3.0 minutes vs. 1.5-2.0 minutes) and lower rates of ascent and descent (0.6-0.7 m/s vs. 0.4-0.5 m/s) than they were during pupping in May and early June. Seals also tended to dive more frequently later in the breeding season (Figure 2.2). The changes in average dive parameters over the course of the breeding season can be mostly attributed to a sharp reduction in the number of deep dives. The average percentage of dives to depths of greater than 20m declined from 40-50% during May to less than 30% in the mating period (Figure 2.3).

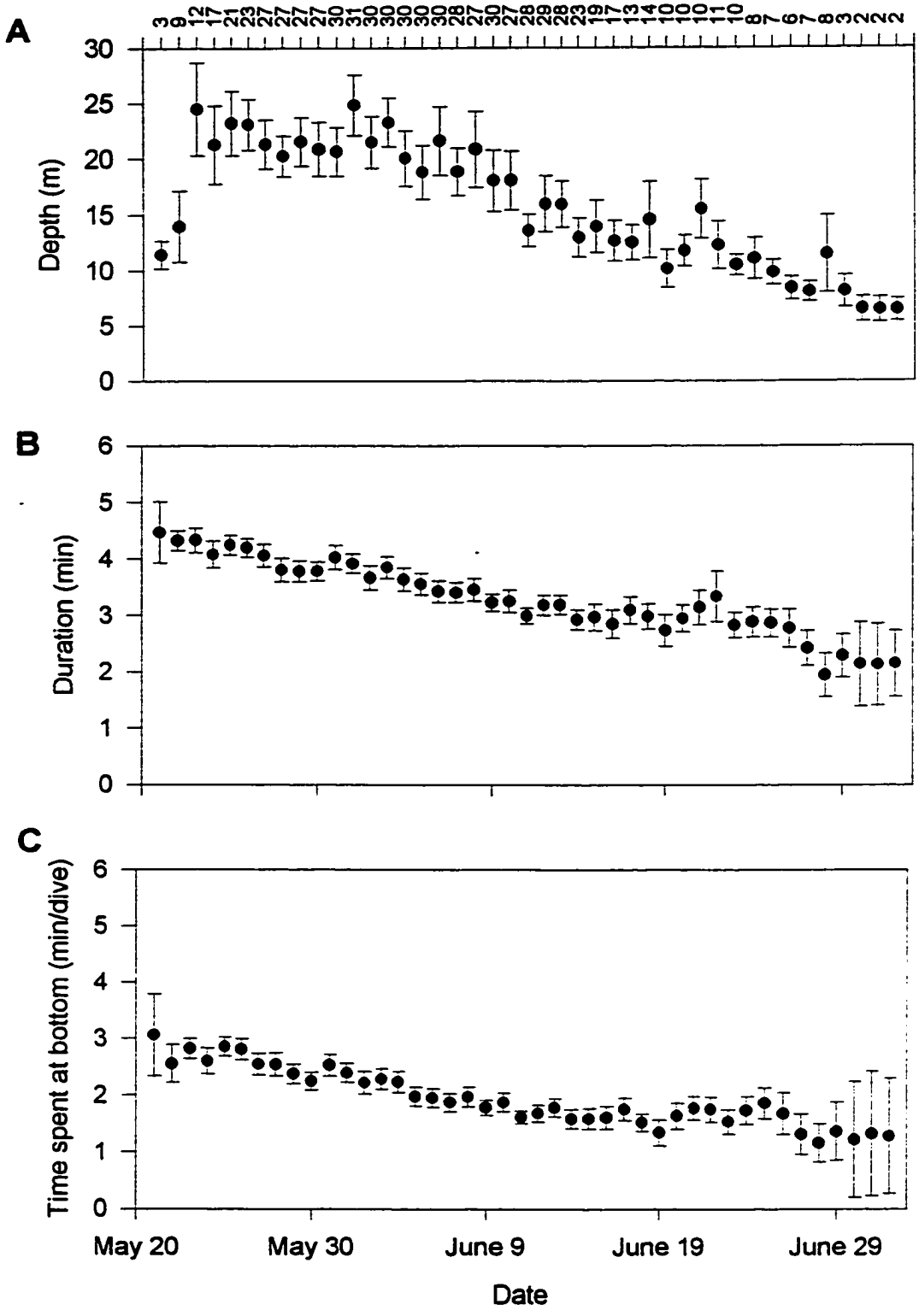


Figure 2.2. Changes in dive characteristics during the breeding season. Changes in mean dive depth (A) duration (B) and bottom time (C) are shown for n male harbour seals indicated at the top of (A) for each day.

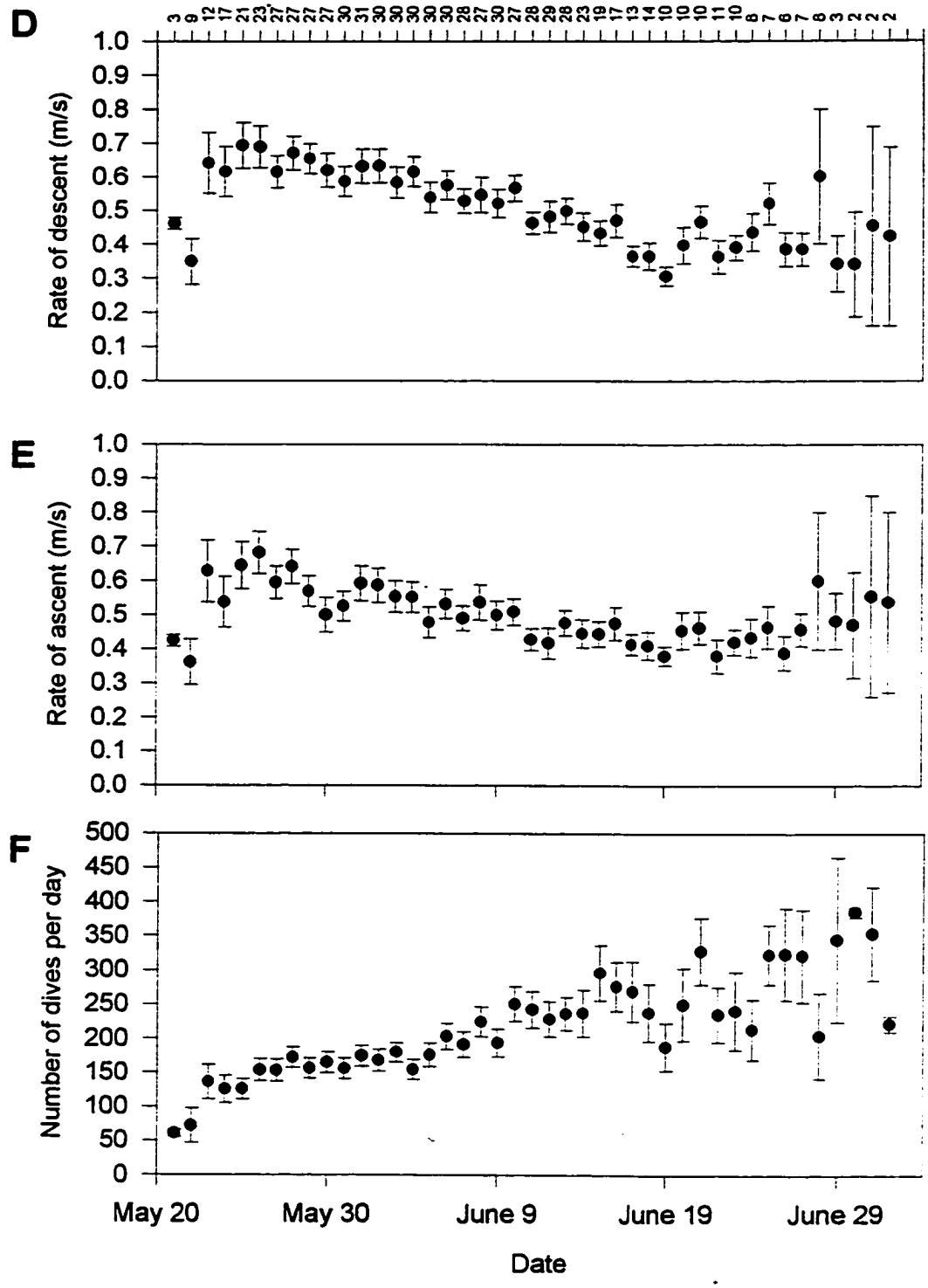


Figure 2.2 (continued). Changes in mean rate of descent (D) ascent (E) and number of dives per day.

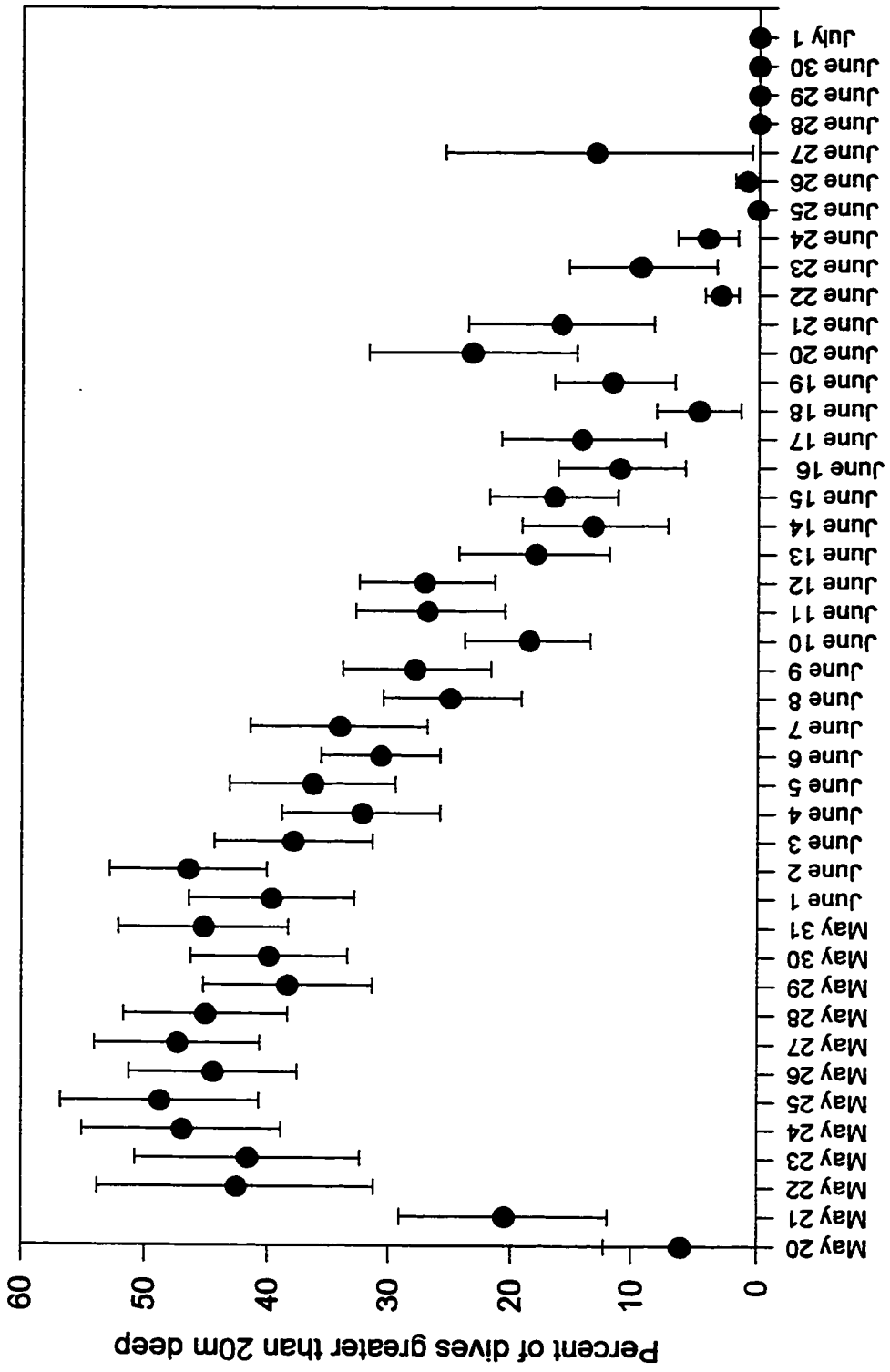


Figure 2.3. Changes in the percent of deep dives during the breeding season.

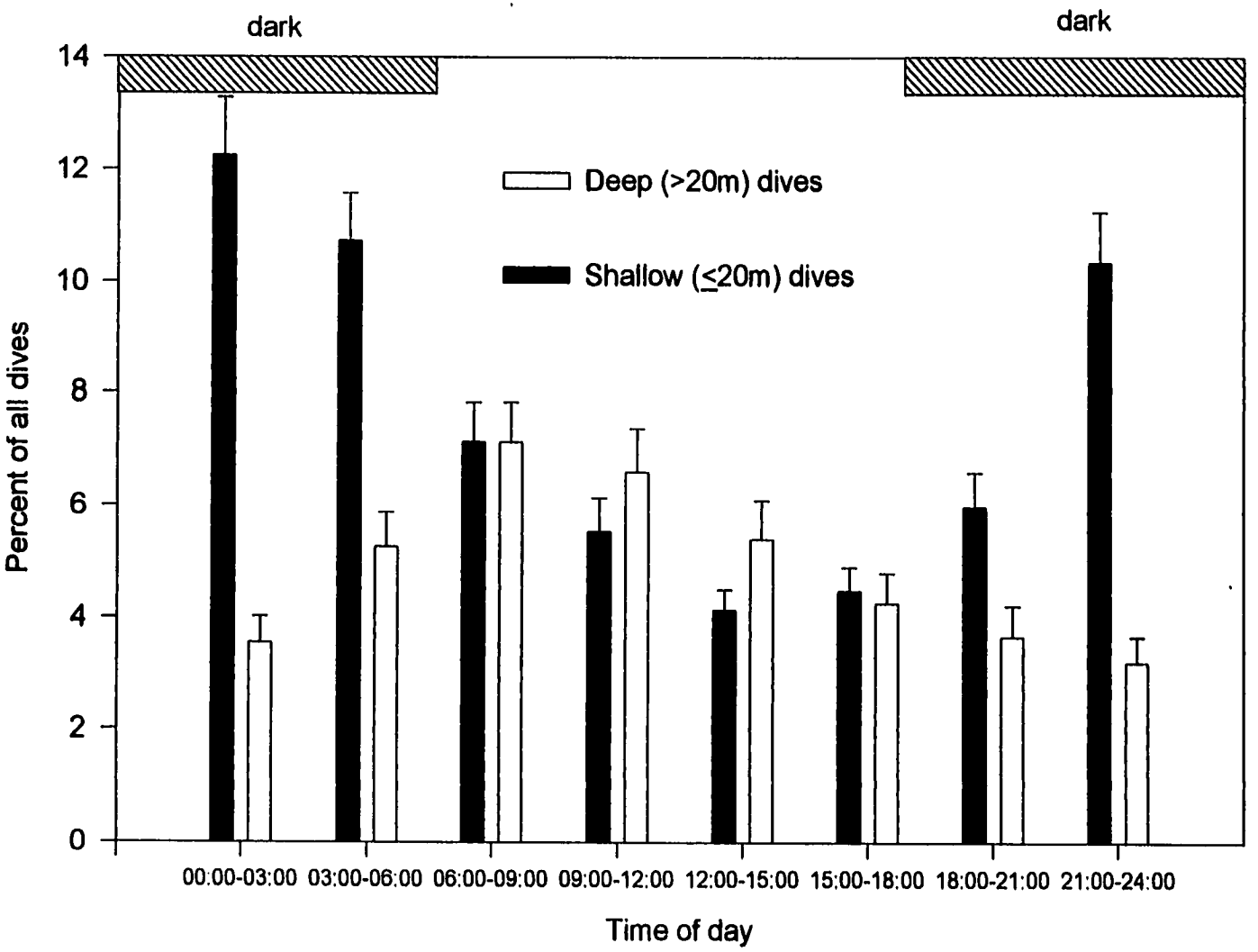


Figure 2.4. Mean percent of deep and shallow dives as a function of time of day. n = 31.

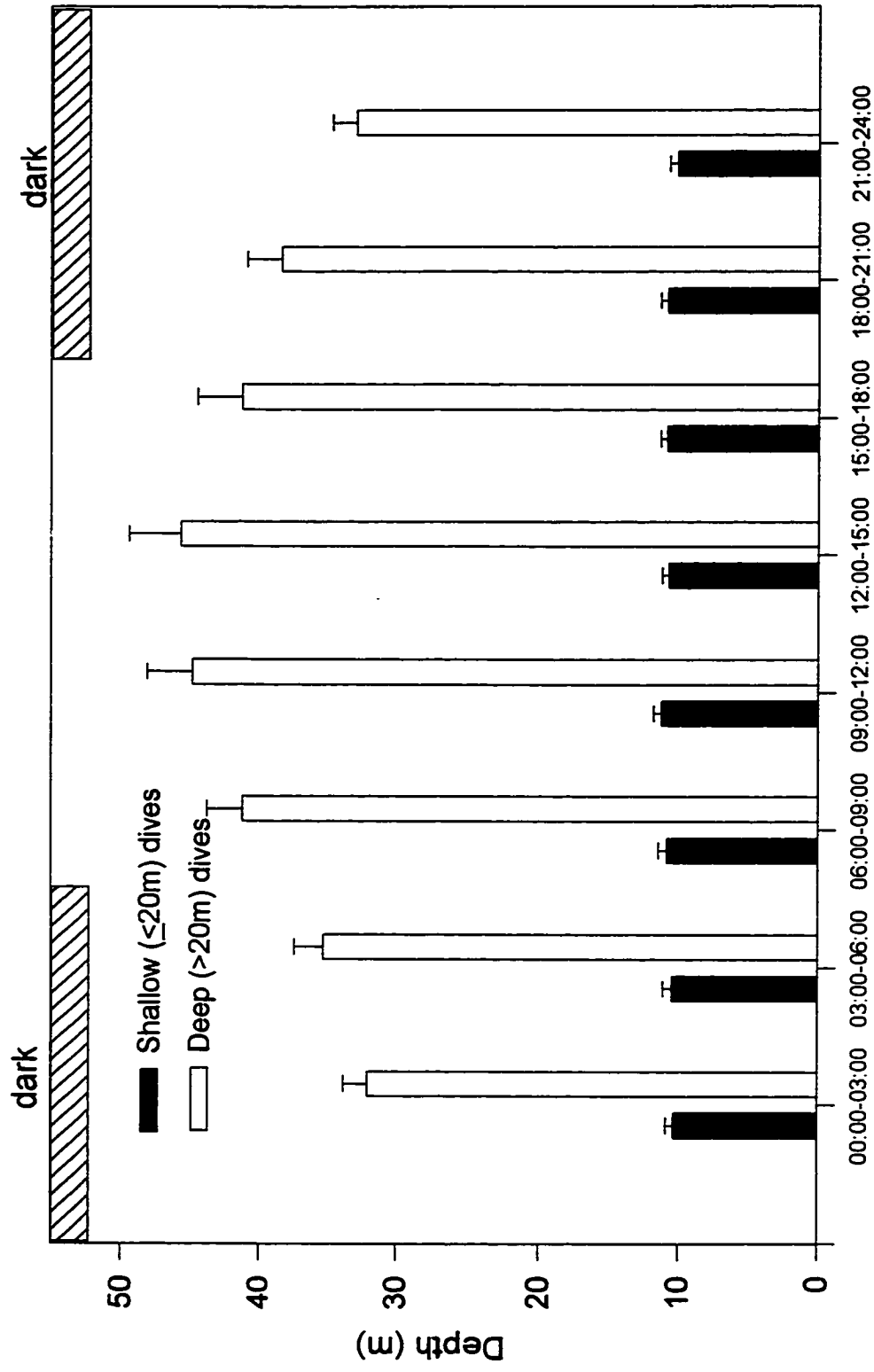


Figure 2.5. Mean depth of dives as a function of time of day. n = 31 males.

To determine whether there was a diurnal pattern in diving activity, as has been reported for Scottish male harbour seals (Thompson **et al.** 1989), the hours of the day were divided into 8 equal blocks (Figures 2.4 and 2.5). The mean percentage of all shallow and deep dives were calculated for each seal for each time block (Figure 2.5), and a two-factor repeated measures ANOVA performed over the eight 3-hour time blocks. The frequencies of deep and shallow dives differed significantly both with time of day (standardized arcsine square root transformed variables; $F = 49.45$; $df = 7, 420$; $P < 0.001$) and from each other ($F=13.268$; $df = 1, 60$; $P < 0.001$). There was a statistically significant interaction between dive depth and time of day ($F=39.48$; $df = 7, 420$; $P < 0.001$), indicating that the diurnal patterns of shallow and deep dive frequencies were significantly different. In general, males made shallow dives more frequently during the dark time periods (21:00-06:00) whereas they made more deep dives during daylight hours (06:00-15:00). The average depth of shallow dives did not vary significantly with time (repeated measures ANOVA $F=0.032$; $df = 7, 210$; $P = 0.86$), however, dives to depths greater than 20m tended to be deeper during the daylight hours (Figure 6) than at night (repeated measures ANOVA; $F = 4.36$; $df = 7, 210$; $P = 0.046$).

Characteristics of Diving Bouts

Graphical representations of dive records (Figure 2.1) indicated that male harbour seal dives tended to cluster into 2 distinct types of bouts distinguished

by maximum dive depth: shallow bouts consisting almost entirely of repeated dives to depths less than 20m, and bouts consisting mainly of considerably deeper dives. When classified by median dive depth, 457 of 642 bouts were thus considered shallow (71.2%) and 185 bouts deep (28.8%). Average characteristics of shallow and deep diving bouts, and the average parameters of dives occurring within deep and shallow bouts are shown in Table 2.3. The characteristics of bout types (bout frequency, dives per bout, time since last bout and bout duration) and of the dives occurring within shallow and deep bouts (average dive depth, duration, bottom time, rate of ascent, rate of descent, and time since last dive) were compared separately using MANOVA. Deep diving bouts differed significantly from shallow bouts overall (MANOVA $F = 11.315$; $df = 4, 53$; $P < 0.001$); they occurred less frequently (univariate ANOVA; $F = 27.77$; $df = 1, 56$; $P < 0.001$), consisted of more dives ($F=32.15$; $df = 1, 56$; $P < 0.001$) and were of longer duration ($F=23.80$; $df = 1, 56$; $P < 0.001$) than shallow diving bouts. The average parameters of dives occurring within bouts also differed significantly overall (MANOVA $F=53.992$; $df = 4, 53$; $P < 0.001$). Dives occurring within deep diving bouts are of longer duration, have longer bottom time, and greater rates of ascent and descent (by univariate ANOVA, $P<0.001$ for all). With the exception of bout frequency, shallow diving bouts appear to be uniformly more variable between males than deep diving bouts, both in the characteristics of the bouts themselves (dives per bout, CV=92.7% and 64.9%; time since last bout, CV=79.5% and 51.8%; bout duration, CV=78.1% and 66.2%

Table 2.3. Characteristics of deep and shallow diving bouts of adult male harbour seals during the breeding season.

	Shallow bouts				Deep bouts			
Parameter	n	Mean	Range	CV (%)	n	Mean	Range	CV (%)
Frequency (bouts per day)	31	0.55±0.06	0 to 1.14	10.7	31	0.23±0.03	0 to 0.70	14.0
Dives per bout	29	194±33	20 to 813	92.7	29	421±51	127 to 1331	64.9
Time since last bout (minutes)	29	717±106	329 to 2782	79.5	29	788±76	67 to 2030	51.8
Bout duration (minutes)	29	804±117	115 to 3162	78.1	29	2293±282	684 to 7202	66.2
Mean dive depth (m)	29	10.4±0.6	5.7 to 17.4	32.3	29	30.1±1.4	21.0 to 57.9	24.9
Mean bottom time per dive (minutes)	29	1.72±0.11	0.46 to 3.38	35.9	29	2.75±0.08	1.92 to 3.63	15.3
Mean dive duration (minutes)	29	3.09±0.11	1.76 to 4.79	20.1	29	4.28±0.08	3.44 to 5.27	10.3
Mean rate of descent (m/s)	29	0.40±0.02	0.24 to 0.63	22.8	29	0.76±0.02	0.54 to 0.93	13.5
Mean rate of ascent (m/s)	29	0.39±0.02	0.23 to 0.67	25.1	29	0.68±0.02	0.51 to 0.87	14.2
Time between dives (minutes)	29	1.70±0.30	0.06 to 57.2	258.6	29	1.24±0.05	0.80 to 1.81	21.2

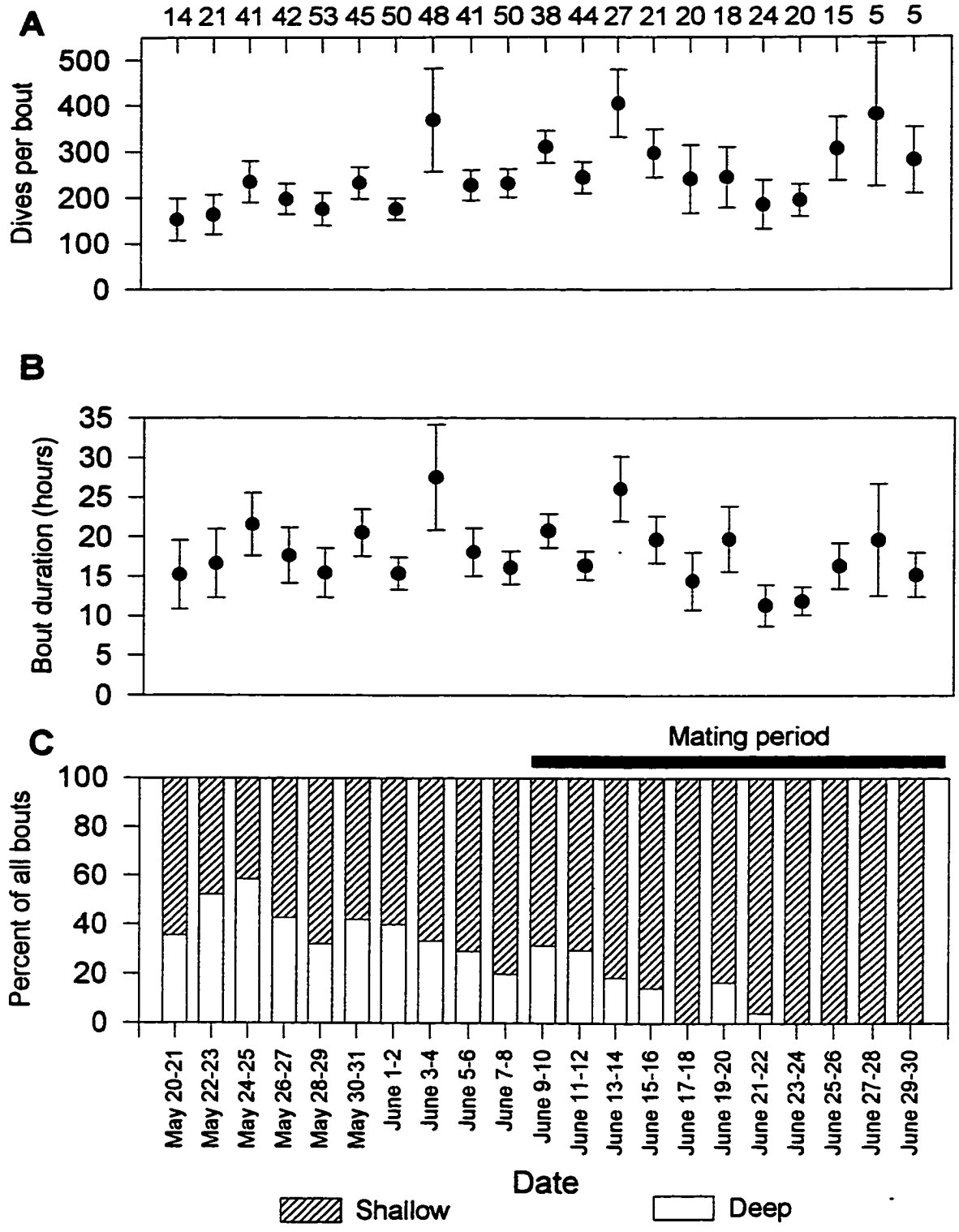


Figure 2.6. Changes in diving bout characteristics over time. Changes in the number of dives per bout (A), mean duration of diving bouts (B) and the percentage of all bouts classified as deep and shallow (C) of 31 male harbour seals. The numbers above (A) indicate the total number of bouts represented in each time interval.

or shallow and deep bouts, respectively) and in all of the average parameters of dives within bouts (see Table 2.3).

Over the breeding season, there were no clear changes in bout duration and the number of dives per bout (Figures 2.6 A and B). However, deep diving bouts occurred more frequently (range 25-60% of all bouts) during the pre-mating period than during the mating period (0-20% of all bouts, Figure 2.6C).

Body Size, Mass Change and Diving Activity

The range of initial masses, from 85 to 131.5 kg, reported here (mean 108.1 $\pm$1.8 kg) is comparable to that reported for adult male harbour seals on Sable Island in previous studies (Godsell 1988; Walker and Bowen 1993a). Rates of mass loss (calculated as the difference between the initial and final mass divided by the deployment interval) were variable between individuals, ranging from 0.02 to 1.05 kg / day (mean 0.46 $\pm$ 0.04 kg / day, CV = 51.2 %). The average mass-specific rate of mass loss for males in this study (calculated as the percentage of initial body mass lost per day) was 0.41 $\pm$ 0.04 % / day (range 0.02 - 0.89 % / day, CV = 47.5 %).

The rate of mass loss was postulated to be related to the following variables: initial body mass (positively), deep diving (negatively) and shallow diving (positively). Preliminary univariate analyses (Figure 2.7) indicated the rate of mass change to be significantly correlated with initial mass ($n = 31$; $P = 0.002$), deep dive effort (following arcsine square root transformation, $r = -0.563$;

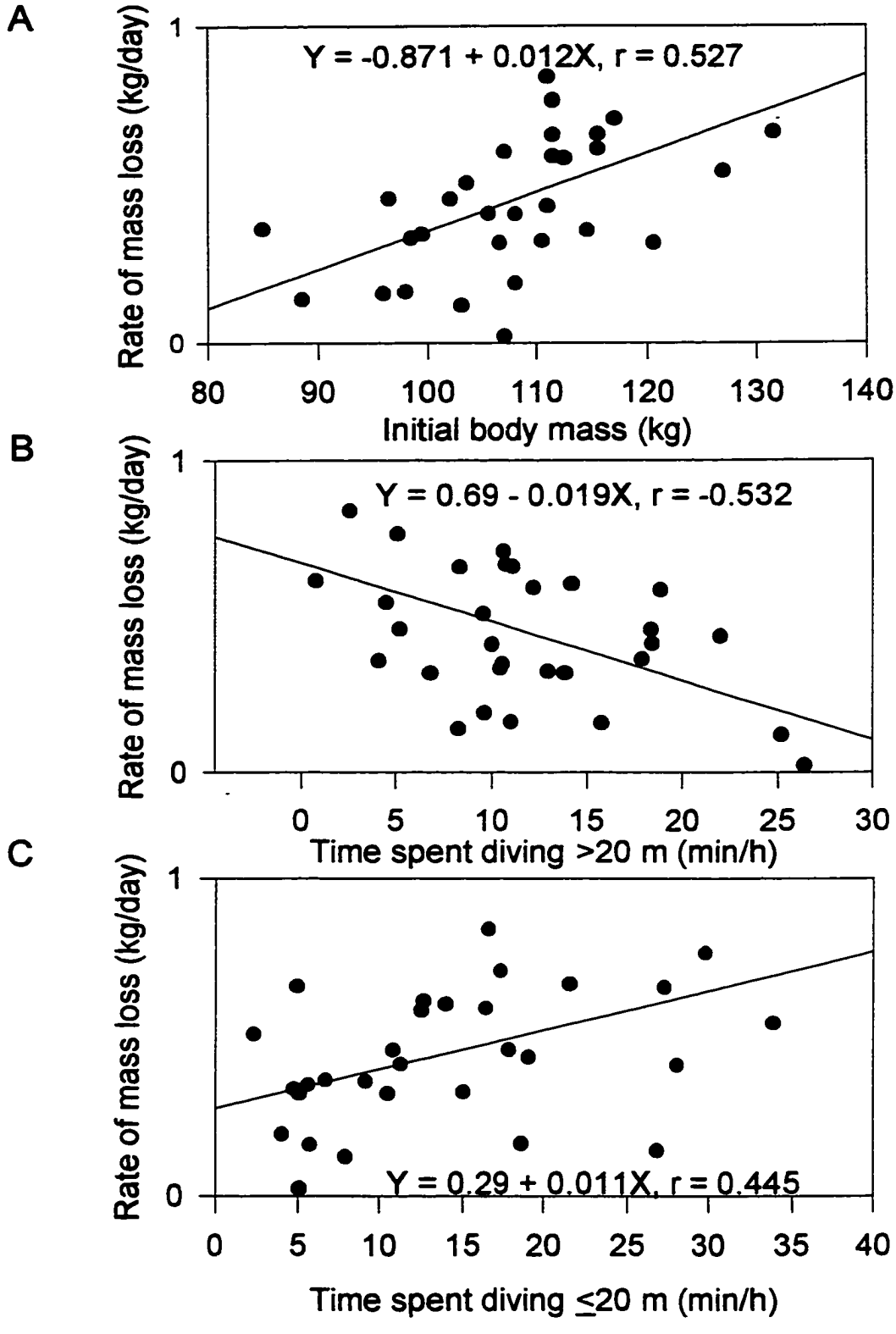


Figure 2.7. Mass loss as a function of initial body mass (A), deep dive effort (B) and shallow dive effort (C) for 31 breeding male harbour seals.

n = 31; P = 0.001) and shallow dive effort (following arcsine square root transformation, r = 0.491; n = 31; P = 0.005). Initial body mass was not significantly correlated with deep dive effort (r = -0.280; n = 31; P = 0.13) nor shallow dive effort (r=0.346; n = 31; P = 0.06). Deep and shallow dive effort were negatively correlated (r = -0.408; n = 31; P = 0.02). I expected the daily rate of mass loss to be correlated with initial mass since resting metabolic rate increases with body size (Peters 1983), so initial mass was included in a stepwise multiple regression analysis on the transformed and standardized dive parameters.

Shallow dive effort was not a significant source of variation when the effects of deep diving and initial body mass were accounted for in the initial multiple regression model (t= -0.84; P = 0.41). Following the removal of shallow dive effort, mass change varied significantly both with deep dive effort (t = 3.13; n = 31; P =0.004) and initial mass (t = -2.79; n = 31; P = 0.009). Together, initial mass and deep dive effort accounted for 46.5 % of the variation in individual rates of mass loss (df = 2, 28; F = 12.16; P < 0.001).

Large seals, those with initial masses greater than the median of 108 kg, were significantly longer than small seals (157.3 ± 1.4 cm vs. 151.4 ± 0.8 cm; n = 31; t = -3.6; P = 0.0016). Large seals lost significantly more of their initial body mass per day, both in absolute terms (two-tailed t-test; t = 4.50; n = 31; P = 0.0001) and relative to their initial body mass (t=3.60; P = 0.0012) over the deployment period (Table 2.4). Over the entire breeding season, large seals

Table 2.4. Characteristics of mass loss and diving activity of relatively large and small male harbour seals during the breeding season.

Parameter	Large males (n=15)	Small males (n=16)
Mass loss (kg day^{-1})	0.61 ± 0.04	0.31 ± 0.04
Percent mass loss (day^{-1})	0.53 ± 0.05	0.31 ± 0.04
Percent deep dives	26.2 ± 5.3	49.8 ± 5.2
Percent deep bouts	22.8 ± 4.9	42.9 ± 6.5
Pre-mating period:		
Hauled-out (minutes $hour^{-1}$)	10.9 ± 1.7	14.5 ± 2.2
Shallow diving (minutes $hour^{-1}$)	14.9 ± 1.8	8.7 ± 1.4
Deep diving (minutes $hour^{-1}$)	12.0 ± 1.8	18.7 ± 2.0
Mating period		
Hauled-out (minutes $hour^{-1}$)	8.6 ± 1.7	16.7 ± 3.3
Shallow diving (minutes $hour^{-1}$)	24.3 ± 3.0	16.1 ± 3.8
Deep diving (minutes $hour^{-1}$)	5.2 ± 2.2	8.9 ± 2.0
Median day of last deep diving bout relative to June 8	0	4

made relatively fewer dives to depths greater than 20m (two-tailed t-test of arcsine square root transformed variables; n = 31; t = 3.19; P = 0.0035) and their records contained fewer diving bouts which were classified as deep (two-tailed t-test of arcsine square root transformed variables; n = 31; t = 2.61; P = 0.014).Repeated measures ANOVA indicated that the overall activity patterns of both large and small males changed with time over the breeding season, and that large and small males differed significantly in proportions of time spent hauled out, shallow diving and deep diving during the breeding season (Table 2.5). Both large and small males spent less time making deep dives in the mating period, but large males spent less time making deep dives and more time shallow diving throughout the breeding season. These differences may be partly attributed to large males changing their overall diving patterns earlier than small males. The median day of the last deep diving bout for large males (8 June, the nominal first day of the mating period) occurred significantly (Kruskal Wallis test, n=31, H = 4.74, P = 0.03) earlier in the season than for small males (12 June, day 4 of the mating period, see Table 2.4). Small males spent more time hauled out during the breeding season than large males, and they increased the time they spent hauled out in the mating period whereas large males spent relatively more time hauled out during the pre-mating period than the mating period.

Table 2.5. Results of repeated measures ANOVA for differences in the activity patterns of large and small male harbour seals over time during the breeding season. Values were subjected to arcsine square-root transformation prior to statistical testing.

Source of variation	F	degrees of freedom	P
Size (large vs. small)	4.32	1, 26	0.048
Time (pre-mating vs. mating)	10.59	1, 26	0.003
Activity (Time hauled out, shallow and deep diving)	3.53	2, 52	0.036
Size by time	0.35	2, 52	0.557
Size by activity	4.78	2, 52	0.012
Time by activity	23.04	2, 52	<0.001

DISCUSSION

These data show that adult male harbour seals spend variable amounts of time diving deeply, presumably on feeding trips, during the breeding season at Sable Island. To reach depths of 50m or greater during these trips, bathymetric maps indicate that males must travel at least 10 km away from the island. These foraging bouts occurred more frequently during the pre-mating period (Figure 2.6). While males were making these foraging trips offshore, females spent most of their time on shore attending their pups (Boness **et al.** 1994). The likelihood of encountering an oestrus female at sea would therefore be low at this time. Thus it appears that many males continue to forage in relatively deep water offshore prior to the mating period to maintain their body mass. and hence their energy stores, until potentially oestrus females start to make foraging trips in late lactation in the second half of June. This conclusion is supported by data from Walker and Bowen (1993a) which indicates that most males maintain or even increase their body mass during the early breeding season.

During the mating period, most males ceased deep-diving and virtually all diving activity occurred at depths of less than 20m (Figure 2.2). The average daily rate of mass loss for male harbour seals in the second half of the breeding season is almost 1 kg/day, representing a mass-specific rate of loss of approximately 0.8% of their initial body mass per day (Walker and Bowen 1993a), a rate similar to that described for fasting male pinnipeds (Deutsch **et al.**

1990; Boyd and Duck 1991). Thus these findings indicate that males cease or substantially reduce feeding during shallow diving bouts. Unlike deep diving activity, shallow dives to the bottom in the second half of the breeding season probably occur within 2 km of the island, given the surrounding bathymetry. In a concurrent study, adult males fitted with VHF transmitters were periodically located at sea throughout the breeding season, and were found to be within 2km of shore significantly more frequently in the latter half of the breeding season (D.J. Boness and W.D. Bowen unpublished data). Evidence from underwater video recordings also shows that during periods of shallow diving activity in the mating period, males are engaged in slow patrolling, chasing and fighting with other males, and stereotypic visual and acoustic displaying in a limited home range near the shore (Boness, Bowen, Marshall and Buhleier unpublished data). During these shallow dive sessions, males both vocalize and exhibit flipper-slapping displays at the surface, perhaps to attract or intercept females in transit through their home range as they depart from or return to the beach from foraging trips or to advertise their presence to other males, or both.

Male diving activity showed a strong diurnal pattern, suggesting that foraging and display behaviours tend to occur at different times of the day (Figures 2.4 and 2.5). Deeper, foraging dives were more common during daylight hours. This is consistent with data from female harbour seal TDRs, as females also tend to make foraging dives to greater depths during the day (Boness **et al.** 1994). Conversely, males made approximately twice as many

shallow dives during the twilight and dark hours (18:00-06:00) than during daylight, suggesting that more behaviour associated with acquiring mates occurs at night or in the twilight hours than during the daytime. The female foraging cycle appears to be daily (Boness **et al.** 1994), so males may be making large numbers of shallow, display dives to maximize their encounter rates during the twilight hours as females may be departing the island in the early morning, and/or returning from foraging trips late in the day or in the early evening.

Larger males may have a mating advantage over smaller males since, with greater energy stores, they can afford to spend less time making foraging trips to offshore locations during the breeding season. Large seals ceased making deep-diving foraging bouts earlier than small seals, and they spent significantly more time in shallow diving activity near the shore through the breeding season. Larger seals may therefore have higher encounter rates with potentially oestrus females as they travel to and from the beach, and by starting to advertise or compete for mates earlier, they may be more likely to establish themselves in a preferential location with respect to predicting female movements at sea. Furthermore, by competing earlier, large males may have access to females which become receptive relatively early in the breeding season, such as non-parturient females which, in captivity, come into oestrus 2 weeks earlier than lactating female harbour seals (Reijnders 1990). Given that males lose up to 1kg per day in the mating period (Walker and Bowen 1993a) when they are displaying, the activity of small seals is probably more

energetically constrained than in larger seals that can expend more energy from their initial body stores in lieu of foraging. Smaller seals may also spend relatively more time resting while hauled out than larger seals. Since more massive seals are also significantly longer than small seals, they are may also represent somewhat older animals, on average. To some extent, the differences in activity between the size groups may thus be related to the effects of experience in addition to the energetic effects of body condition and general body size.

The small body size of harbour seals relative to other pinnipeds has been postulated as important in the evolution of their maternal strategies (Bowen **et al.** 1992). Larger-bodied phocid seal females, such as the grey and elephant seals, can support the entire cost of lactation from their initial body stores, and hence can afford to remain onshore continuously throughout parturition, nursing, weaning and mating. Boness **et al.** (1994) argued that the constraints of small body size of harbour seals have resulted in the evolution of a maternal foraging cycle, similar to that of otariid females. Since females are forced to spend increasing amounts of time in the water making foraging trips to regain energy in late lactation as they approach oestrus, small body size may have also played a role in maintaining aquatic copulation in this species. The results of this study suggest that energetic constraints arising from small body size may have also played a role in the evolution of male reproductive strategies. At Sable Island, most males forage during the early stages of the breeding season, then switch to

energetically expensive reproductive displays in relatively shallow water near the haul-out when they can afford to do so, and when it should pay off in terms of mating success.

CHAPTER III

THE ENERGETICS OF REPRODUCTION IN MALE HARBOUR SEALS

INTRODUCTION

Studies of the energetics of reproduction in pinnipeds have traditionally focused on females (Fedak and Anderson 1982; Costa **et al.** 1986;Costa and Gentry 1986; Costa **et al.** 1989; Bowen **et al.** 1992; Oftedal **et al.** 1993; Iverson **et al.** 1993). Fewer studies have investigated the energetic costs of reproduction in male pinnipeds, mostly on polygynous, terrestrially mating species such as grey seals, *Halichoerus grypus*, (Anderson and Fedak 1985; Tinker **et al.** 1995), northern elephant seals, *Mirounga angustirtostris*, (Deutsch **et al.** 1990) and Antarctic fur seals, *Arctocephalus gazella*, (Boyd and Duck 1991). Since male pinnipeds provide no parental care, their reproductive effort is directed solely towards procuring mates (Trivers 1972). Males in terrestrially mating species males generally fast as they remain onshore throughout the breeding season. Consequently their energetic investment in reproduction can be measured indirectly by monitoring mass loss (Deutsch **et al.** 1990; Tinker **et al.** 1995), and more accurately by estimating changes in body composition and water flux by isotope dilution (Boyd and Duck 1991). Some of these studies have demonstrated a positive correlation between the energetic investment in

reproduction and mating success, based on observed copulations (Anderson and Fedak 1985; Deutsch **et al.** 1991; Tinker **et al.** 1995).

Few studies have addressed the energetic costs of reproduction for males in aquatically mating pinniped species. Most species of the family Phocidae (or the true seals) mate aquatically (Stirling 1983; LeBoeuf 1991), hence this represents a major gap in our understanding of the energetics of male reproduction among pinnipeds. Since most aquatically mating seal species are relatively small-bodied and less size-dimorphic than terrestrially mating pinnipeds (Stirling 1983), males generally have smaller absolute stores of body energy to draw upon during the mating season, and thus are more limited in their ability to fast than those of larger species. Energetics may therefore determine male reproductive behaviour and shape the mating systems of aquatically copulating pinnipeds. For such species, data on energetics and activity during the breeding season are difficult or impossible to obtain since males largely stay in the water where they cannot be directly observed. Also, they are often difficult to capture such that the necessary longitudinal measurements of body mass, composition and water flux have been limited to a few individuals (Reilly and Fedak 1990).

Harbour seals mate in the water, and previous studies monitoring longitudinal changes in mass and circulating chylomicrons (Walker and Bowen 1993a), diving behaviour (Chapter II) and locations at sea (Van Parijs **et al.** 1996) have shown that males reduce or perhaps even discontinue feeding during the breeding season. Unlike terrestrially mating seals, which must fast

while on shore during the breeding season, male harbour seals can feed every time they enter the water, so estimates of reproductive effort from mass losses alone will not usually reflect the total energetic cost of reproduction. In previous studies of diving behaviour (Chapter II; see also Walker and Bowen 1993a), we found that male harbour seals lost mass at a greater rate during the latter portion of the breeding season, when they made shallow dives associated with mating, whereas they tended to maintain their mass by foraging during periods of extended deep diving earlier in the breeding season. Male harbour seals thus appear to make a trade-off between time spent foraging and time spent trying to obtain mates while at sea. However, without knowledge of changes in body composition and water flux, changes in body mass provide only minimum estimates of energy expenditure and the extent to which animals are feeding.

To date, one study has employed isotope dilution methods to monitor changes in body composition and water turnover in an aquatically mating pinniped. Reilly and Fedak (1991) measured changes in the body composition, water flux (n = 3) and metabolism (n = 1) of male harbour seals and found them to be in a state of negative energy balance during the breeding season. Their data from the one individual given doubly-labelled water also suggested that aquatic mating was extremely costly, since the estimated mass-specific metabolic rate of this individual was six times the basal rate predicted by Kleiber's (1975) equation. However, the conclusions of that study must be considered tentative given the small sample.

The purpose of this study was to quantify changes in body composition and water flux of male harbour seals during the breeding season using hydrogen isotope dilution in relation to their activity patterns while at sea. Changes in body mass and body composition provide an accurate assessment of the net energy invested in reproductive effort from initial body stores, and water flux enables an estimate of food intake during the period of study. Together, data on changes in mass, body composition, and water flux can be used to describe the total energy expended during the breeding season. I also examined initial body size and body composition of male harbour seals, reflecting the amount of stored energy individuals possess at the beginning of the breeding season, as factors influencing their reproductive behaviour.

METHODS

Study site and animals

The study was conducted during the breeding seasons (mid-May to the end of June) of 1992, 1993, and 1994 on the north beach of Sable Island (43°55'N; 60°00'W), a partially vegetated sandbar 160 km east of Halifax, Nova Scotia. Between 300 and 500 harbour seal females gave birth on Sable Island during each year of the study.

Male harbour seals were captured, individually marked, and weighed as described by Walker and Bowen (1993ab) near the beginning of each breeding season (range 20 May - 30 May in each year). Twelve (1992, 1993) or 13 (1994) males were selected for deuterium oxide (D_2O) dilution in each season. I designed the study to represent a broad range of initial body masses, as previously reported for adult male harbour seals in this population of 85-130 kg (Godsell 1988; Walker and Bowen 1993ab). I repeated isotope administration near the end (15 June - 30 June) of the breeding season to estimate the total change in body composition. All males in 1993 and in 1994 were also recaptured twice at 7-10 day intervals between the initial and final equilibration to monitor changes the rate of mass loss. I administered sufficient isotope to a subset of these animals (n = 12 in 1993; n = 6 in 1994) at the initial equilibration to monitor the changes in D_2O concentration throughout the breeding season. A blood sample was taken from these 'water flux' animals during every recapture.

In 1993 and 1994, the first isotope administration and first recapture occurred prior to the appearance of weaned pups in the colony, while the

second and the third recaptures (final isotope administration) occurred following the appearance of weaned pups. Since harbour seals are believed to mate near the time of weaning (Boulva and McLaren 1979; Thompson 1988), I divided the breeding season into a pre-mating period and a mating period. On Sable Island, the first pups were born on 15 May, 13 May, and 14 May in 1992, 1993, and 1994, respectively. Assuming a mean lactation duration of 24 days (Muelbert and Bowen 1993), oestrus females would have become increasingly numerous after about 8 June in each year (i.e., the median expected date of the appearance of the first weaned pup). I used this date to divide the breeding season into the pre-mating and mating periods. Changes in body mass, composition, and rates of water flux calculated between the first 2 measurements are referred to as having occurred during the pre-mating period, and between the last 2 measurements as during the mating period.

Males were also fitted with a TDR at the time of initial isotope administration (Chapter II). I use data describing deep (to depth > 20m) and shallow (20m or less) dives of males, as presented in Chapter II, to investigate links between food intake, gross energy balance, and the activity of individual males at sea.

Isotope administration and analysis

Once secured in nets, seals were sedated with diazepam (approximately 0.2 mg per kg body mass) to minimise stress during handling and isotope

administration. A pre-weighed quantity of deuterium oxide (D_2O, 99.8% purity, Stable Isotopes Division, ICN Biochemicals, Cambridge, Mass.) was administered by syringe with a number 12 French gastric tube, at a dose of approximately 0.6 g/kg body mass for determining body composition, or at 2 g/kg body mass to animals which were in the water flux study. Syringe and tube were flushed twice with 5 mL quantities of distilled water, followed by 50 mL of air, to ensure complete isotope delivery. Males were held in pens for 2.5 to 4 h after isotope administration to allow equilibration. Seals were then bled twice from the extradural vein with 30 minute intervals between bleedings to determine if equilibration of the isotope had occurred. Blood samples were also taken during recaptures of water flux study animals to measure D_2O remaining in the animal, and a single blood sample was taken from each animal prior to the final administration of isotope to determine residual D_2O concentration.

Blood samples (8 cm^3) were collected without coagulants and centrifuged within 24 h of collection to separate sera. Sera were transferred to cryovials and stored at -20 °C prior to heat distillation and assay for D_2O concentration with infrared spectrophotometry following the method of Oftedal and Iverson (1987).

Estimation of body composition and water flux

All concentrations of D_2O were corrected by subtracting the natural background concentration of D_2O found in male harbour seals on Sable Island

(0.011 ± 0.001 %, n = 6). I considered an animal to have equilibrated the administered isotope if the concentration of D_2O in the second sample did not exceed the first more by than 0.01 %. At equilibration, body water pool (BW) was then estimated as:

(1) $$BW\ (kg) = M_d / (10 \times S_d)$$

where M_d is the amount of D_2O administered (g) and S_d is the average percentage of D_2O at equilibration. Since the dilution space of D_2O has been found to overestimate total body water, I corrected BW by 2.8% to estimate TBW (Reilly and Fedak 1990). Given that data are not available for harbour seals, body composition was calculated from TBW and total body mass (TM) using regression equations derived by Reilly and Fedak (1990) for grey seals to give total body fat (TBF), total body protein (TBP) and total body energy (TBE). The equations used were:

(2) $$TBE\ (in\ MJ) = (40.8 \times TM) - 0.4 - (48.5 \times TBW)$$

(3) $$\%\ TBP = (0.42 \times \%\ TBW) - 4.75$$

(4) $$\%\ TBF = 105.1 - (1.465 \times \%\ TBW)$$

We calculated the fractional daily water flux (k) from changes in D_2O concentration over time (see Figure 3.1) using calculations described by Oftedal and Iverson (1987). The concentration of D_2O in a given sample was corrected for changes in pool size by assuming that changes in TBW were linear during the study period, to give a concentration of D_2O (C_t) corrected for the change in TBW at time t. Regression of Ln (C_t) over time gives an estimate of k over the entire breeding season (Figure 3.1). To determine if k changed as the breeding season progressed, independent two-point estimates of k were also made for the pre-mating period (k_p), as the rate of change between the initial equilibration and the first recapture, and for the mating period (k_m), as the rate of change between the third and the final recapture (see Figure 3.1).

Estimates of total daily water flux for the entire breeding season (TWF), the pre-mating (TWF_p), and mating periods (TWF_m) were made for each seal from the appropriate k and the mean daily change in TBW (ΔTBW) as:

$$TWF = k \times TBW_{1/2} + \Delta TBW \quad (5)$$

where $TBW_{1/2}$ represents the estimated total body water at the midpoint of the period in question.

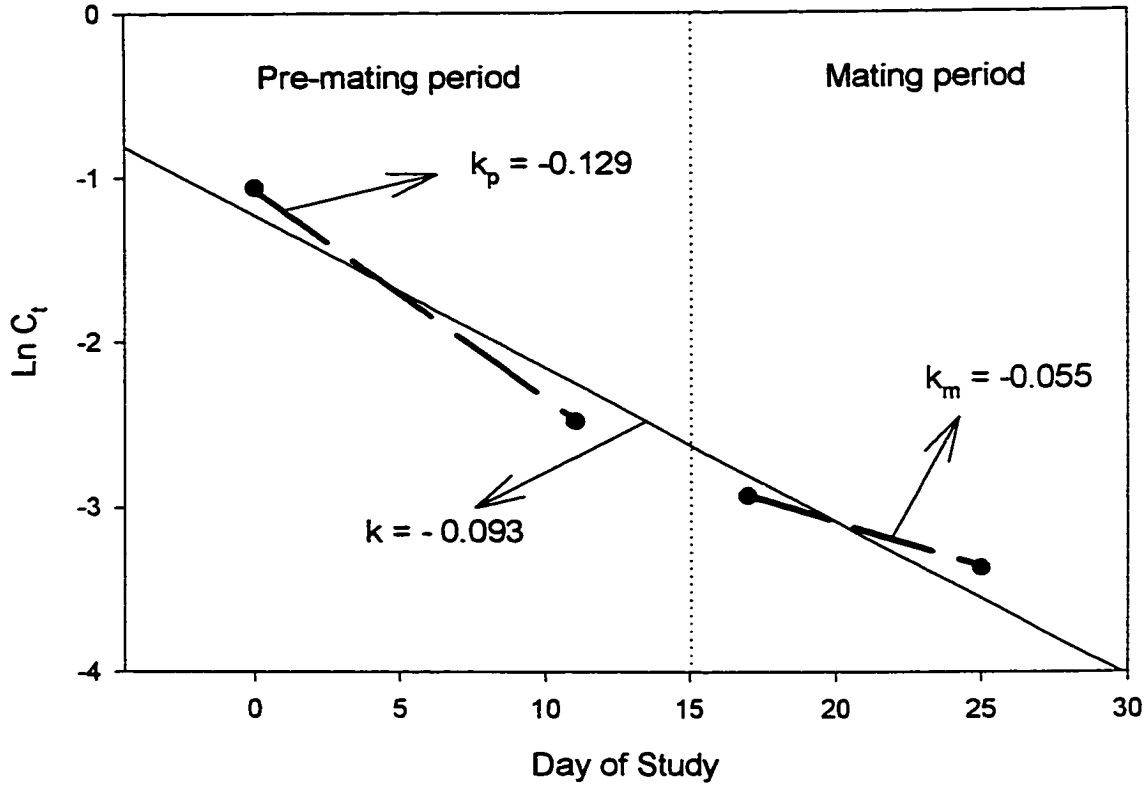


Figure 3.1. Changes in D_2O concentration in a male harbour seal over time.

Estimation of metabolic water production, food intake and total energy expended

I estimated the rate of food intake for males from TWF assuming the following relationship:

(6) $$TWF = FWI + MWP$$

where FWI represents total water intake from feeding and MWP represents metabolic water production estimated from field metabolic rate (FMR). Without using doubly-labelled water, estimates of FMR can only be made from data describing water flux or changes in the TBE of fasting pinnipeds (Costa and Gentry 1986; Boyd and Duck 1991). I was never sure of the feeding status of these animals as male harbour seals enter the water every day. Hence, for the purpose of estimating FWI I estimated FMR from daily changes in TBE and mass loss, under the assumption that harbour seals would lose 1% of their initial body mass per day if they were fasting during the breeding season. This assumption was based on previously published mass change data from fasting juvenile harbour seals (Markussen and Ryg 1992- 1.06%/day) and published data on mass loss in other fasting, breeding adult male pinnipeds of similar body size such as grey seals (Anderson and Fedak 1987- 0.97%/day; Tinker **et al.** 1995- 0.92%/day) and Antarctic fur seals- (Boyd and Duck 1991- 0.81%). In addition, both Walker and Bowen (1993a) and Reilly and Fedak (1991) have

reported maximum rates of mass loss of approximately 1.0 % per day for breeding male harbour seals.

FWI was then converted to food intake (FI) assuming a diet consisting of sandlance (*Ammodytes* sp.); the major prey at this time of year (Walker and Bowen 1993a; W.D. Bowen, unpublished data), composed of 78.44 % H_2O, 5.14% fat and 15.47% protein (S. J. Iverson, unpublished data), and assuming the catabolism of 1g of fat yields 1.071 g H_2O and 1g of protein 0.396 g H_2O (Schmidt-Nielson 1979). FI was converted to total food energy ingested assuming 1 kg of sandlance contains 4.80 MJ of gross energy from the catabolism of 51.4 g of fat at 39.3 kJ/g and 154.7 g of protein at 23.6 kJ/g (Schmidt-Nielson 1979). I converted total food energy ingested to daily food energy intake (FEI) assuming the metabolizable energy available from dietary input was 85%; based on estimates of metabolizable energy available to grey and harp seals fed herring ranging from 82.7 to 88.7 % (Ronald **et al.** 1984; Keiver **et al.** 1984). Total energy expended per day (TEE) was then calculated as the sum of the negative change in daily body energy (ΔTBE) plus FEI:

$$TEE = -\Delta TBE + FEI \quad (7)$$

We calculated TEE for the entire breeding season using ΔTBE and FWI; and also for both the pre-mating (TEE_p) and mating (TEE_m) periods similarly using the independently derived two-point estimates of TWI_p and TWI_m, and ΔTBE_p and ΔTBE_m, respectively.

Data Analysis

All data are presented as the mean plus or minus one standard error. The probability of type I error was set at $\alpha = 0.05$ for all statistical tests. Prior to statistical tests, all data were checked for normality and transformed if necessary. Arcsine square-root transformation was used on proportions prior to statistical testing, but regression equations and means are presented for the untransformed data. ANCOVA models were used in preference to ratios or percentages to account for body size effects when testing hypotheses using energetic variables. Initial, final or average mass ([initial + final mass]/2) was used as a covariate where appropriate. Statistical tests involving energetic parameters which may scale allometrically (i.e. relations between morphometric variables) were fitted using a reduced major axis regression model to account for measurement error in both variates (Rayner 1985). Statistical analyses were performed with Minitab except for repeated measures ANOVA models which were tested in SPSS.

RESULTS

Changes in body mass and composition

Initial TBW could not be calculated for 2 of the 37 study animals (one in 1992, one in 1993) since the isotope was not equilibrated. One of these seals was a subject in the water flux study, and was omitted from those analyses. I obtained at least 4 captures on all other water flux study animals (n = 17). Final equilibrations were not performed on 3 additional animals in 1992 due to logistical constraints and I failed to recapture 1 male in 1994. In total, I obtained mass change data from 34 individuals, body composition change data from 31 individuals and water flux data from 17 individual males. TDR records were recovered from 30 animals, 14 of which were in the water flux study.

TBW was strongly correlated with mass at both the initial and final equilibrations (Figure 3). In both instances the slope of the standardized variables estimated from reduced major axis regression was not significantly different from 1 (95% confidence interval = [0.81, 1.235] and [0.679, 1.48] for initial and final equilibrations, respectively), thus body composition was independent of initial and final body mass. ANCOVA indicated that there were significant differences in both initial and final TBW between years after the influence of body size was accounted for (initial or final mass included as covariate, F_{year} = 10.01 and 4.39; df = 2,31 and 2,27; $P < 0.05$ for initial and final TBW, respectively). Since initial isotope administrations were performed at the same stage of the breeding season in each year (range 20 May-30 May),

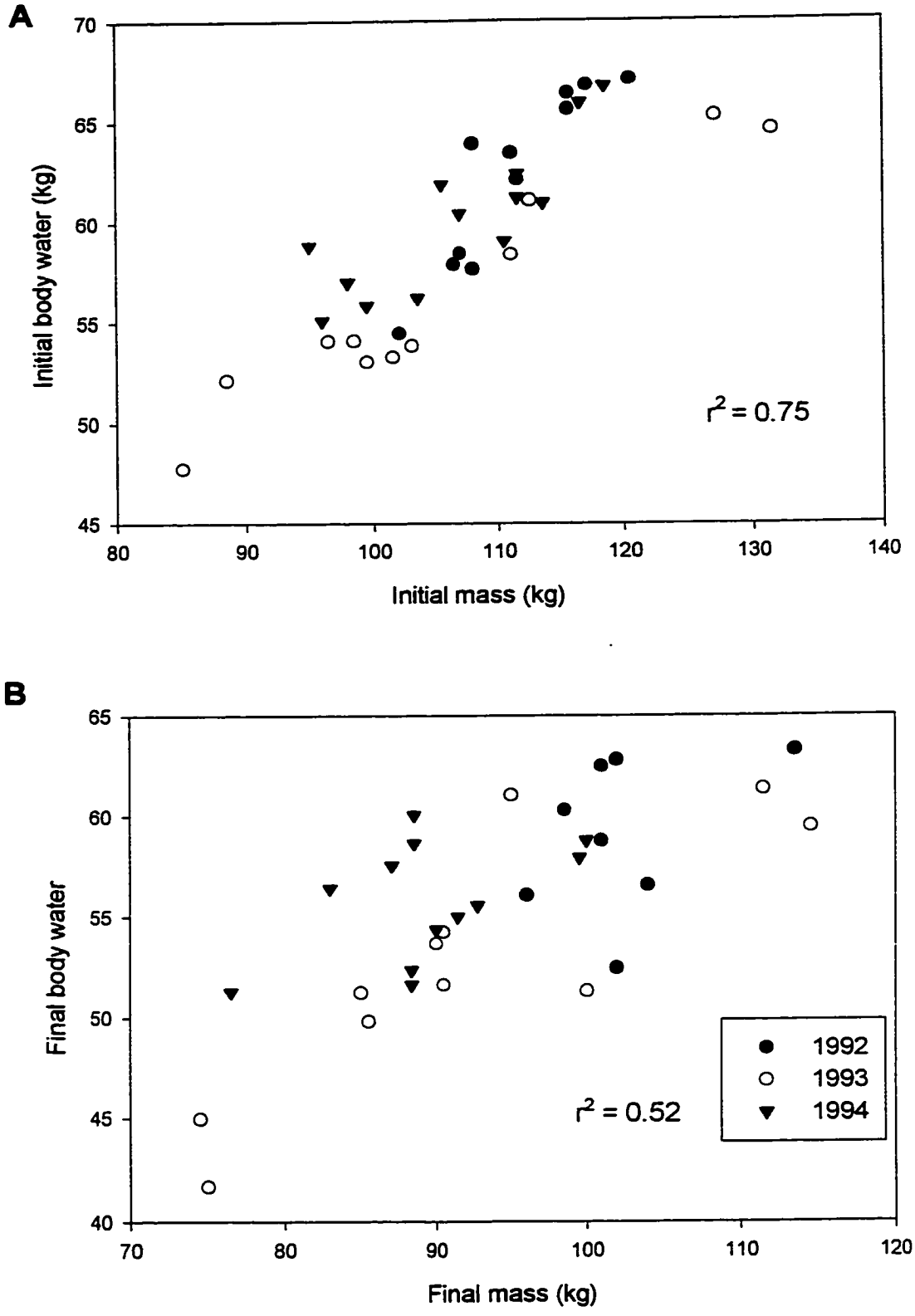


Figure 3.2. Total body water versus body mass. Data from the initial (A) and final (B) weighings and equilibrations are shown.

concurrently with the period of peak pupping in the colony (W.D. Bowen unpublished data), the ANCOVA result indicates that males sampled in 1993 were composed of relatively less TBW at the start of the season. This indicates that males in 1993 had significantly higher percent fat, and lower protein content than in 1992 or 1994 (Table 3.1) at the beginning of the breeding season. This difference can be attributed to the influence of the two largest males in the 1993 sample (see Figure 3.2A) which had low TBW content relative to their initial mass. There were no significant differences in initial percent fat or protein body composition between years if these two points are removed. Neither average initial mass nor initial TBE varied significantly between years.

Final equilibrations were performed significantly later in each successive season (22.4 ± 0.2, 27.6 ± 3.3, and 33.8 ± 0.7 days after the initial equilibration in 1992, 1993 and 1994, respectively; one-factor ANOVA, $F=29.8$; $df=2,31$; $P<0.001$) such that final body mass and composition data could not be statistically compared between years. ANCOVA results indicated that males sampled in 1994 had higher TBW content relative to their body mass at the final equilibration, therefore lower percentage fat content (Table 3.1), but given the later final equilibrations in 1994 this is not surprising. Rates of change in mass, fat, protein and TBE did not differ significantly between years, therefore data from all years were pooled for all subsequent analyses.

Initial masses ranged from 85.0 to 131.5 kg. Initial fat and protein content ranged from 14.1 to 32.9 % and 15.9 to 21.3 %. Fat content was more variable

Table 3.1 Changes in mass, body composition, and total body gross energy of male harbour seals during the breeding season.

Variable	Year	Initial	Final[1]	Rate of change (per day)
n	1992	11	8 (11 for mass)	8 (11 for mass)
	1993	11	11	11
	1994	13	12	12
	total	35	31 (34 for mass)	31 (34 for mass)
Mass (kg)	1992	111.1 ± 1.7	102.3 ± 1.8	-0.36 ± 0.07
	1993	105.0 ± 4.4	92.0 ± 3.9	-0.45 ± 0.07
	1994	106.7 ± 2.2	89.5 ± 1.8	-0.53 ± 0.08
	average	107.5 ± 1.7	94.7 ± 1.8	-0.47 ± 0.04*
Percentage Fat	1992	22.9 ± 0.9	20.0 ± 2.0	-0.16 ± 0.06
	1993	26.1 ± 1.2	20.4 ± 1.7	-0.20 ± 0.05
	1994	22.1 ± 0.9	13.2 ± 1.7	-0.26 ± 0.05
	average	23.6 ± 0.6**	17.5 ± 1.2	-0.21 ± 0.03*
Percentage Protein	1992	18.7 ± 0.2	19.6 ± 0.6	+0.05 ± 0.02
	1993	17.8 ± 0.3	19.4 ± 0.5	+0.06 ± 0.01
	1994	19.0 ± 0.3	21.5 ± 0.5	+0.08 ± 0.01
	average	18.5 ± 0.2**	20.3 ± 0.3	+0.06 ± 0.01*
Total body energy (MJ)	1992	1518 ± 27	1305 ± 78	-10.6 ± 3.1
	1993	1559 ± 103	1193 ± 93	-13.0 ± 2.4
	1994	1435 ± 54	944 ± 66	-14.8 ± 2.4
	average	1500 ± 39	1126 ± 53	-13.1 ± 1.5*

1) Final measurements made 22.4 ± 0.2, 27.6 ± 3.3 and 33.8 ± 0.7 days after the initial measurement in 1992, 1993 and 1994, respectively.
* indicates rate of change significantly different from 0 (one-sample t-test; $p<0.001$ for all)
** indicates significant difference between years (one-factor ANOVA; df 2,32; $P<0.05$ for all)

among males than protein (CV = 15.7 vs. 5.7% for fat and protein, respectively). Initial TBE ranged from 1022 MJ to 2233 MJ and was more variable among males than initial mass (CV = 15.3 % vs. 9.3 %). The most massive seal had the highest TBE content (2233 MJ), but there were 2 heavier males with lower TBE than the smallest individual (85 kg). Rates of mass loss varied greatly among males, ranging between 0 and 1.05 kg/day (CV = 50.6 %) with a maximum rate relative to initial body mass of 0.90 % per day. Males lost up to 33.5 kg over the entire breeding season, and the maximum percent of initial mass lost was 28.8 % by a male in the 1994 sample over 32 days. Changes in TBE were even more variable among males, ranging from -28.3 to ± 4.2 MJ/day (CV = 59.4%). One individual in the 1992 sample slightly gained TBE during the study period (4.2 MJ/day) and maintained constant mass, all other males were in negative net energy balance during the study period. Fat, protein, and water accounted for 64.3 ± 4.8, 6.9 ± 1.4 and 27.8 ± 3.3% of mass loss, respectively (n = 31). Assuming an energy density of 39.33 MJ/kg for the energy content of fat, fat catabolism accounted for 89.4 ± 3.4% of the total change in TBE.

Water flux, activity and total energy balance

The daily change in TBE (ΔTBE) was positively correlated with deep diving effort (Figure 3.3) and negatively correlated with shallow diving effort (r = -0.346, data not shown). Following arcsine square-root transformation of deep and shallow dive effort, only the relationship between ΔTBE and deep dive effort was statistically significant ($F=10.7$; df =1, 25; $P=0.003$), indicating that seals

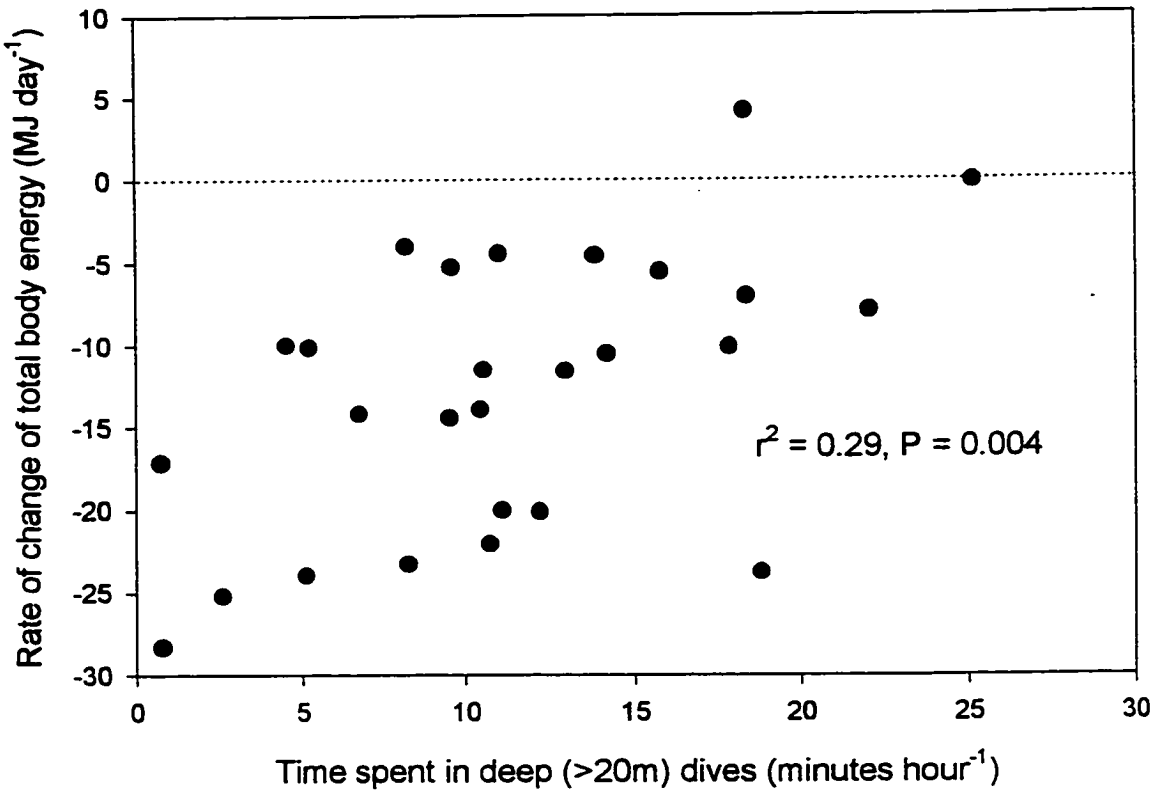


Figure 3.3. Change in total body energy versus time spent deep diving for 27 male harbour seals.

which spent relatively more time diving deeply lost less energy from their initial body stores over the breeding season.

Expressed in relative terms, male harbour seals lost on average 0.41 ± 0.04% of their initial body mass per day over the entire breeding season. ΔTBE was closely correlated with daily percent mass loss (Figure 3.4). Assuming a seal losing 1.0% of its initial mass per day was fasting, for the purpose of estimating MWP the estimated FMR was 33.2 MJ per day (ΔTBE = 2.15 + 35.3[rate of % mass change], see Figure 3.4).

I then assumed that male harbour seals produced approximately 0.9 kg of water per day (MWP) entirely from lipid catabolism (FMR = 33.2 MJ at 0.0272 kg H_2O /MJ; Schmidt-Nielson 1979). Estimates of TWF for all of the animals in this study exceeded 0.9 kg per day, so I used the difference between TWF and MWP as a measure of water intake from feeding (FWI, equation [6]). Assuming a diet consisting entirely of sandlance, FEI ranged between 13.6 and 33.0 MJ/day (CV = 25.7%), giving an average TEE for adult male harbour seals during the breeding season of 32.1 ± 2.1 MJ/day by equation (7) (Table 3.2). TEE ranged between 17.7 and 51.2 MJ/day (CV = 25.7%). The mean estimate of TEE was not significantly different from the FMR estimate (33.2 MJ/day) we used to estimate MWP ($t = -0.54$, $n = 17$, $P = 0.60$). Expressed in terms of mass-specific power, the average TEE is the equivalent of 3.79 ± 0.23 W/kg. TEE was not significantly correlated with average body mass ($r = 0.335$, $n = 17$, $P = 0.189$).

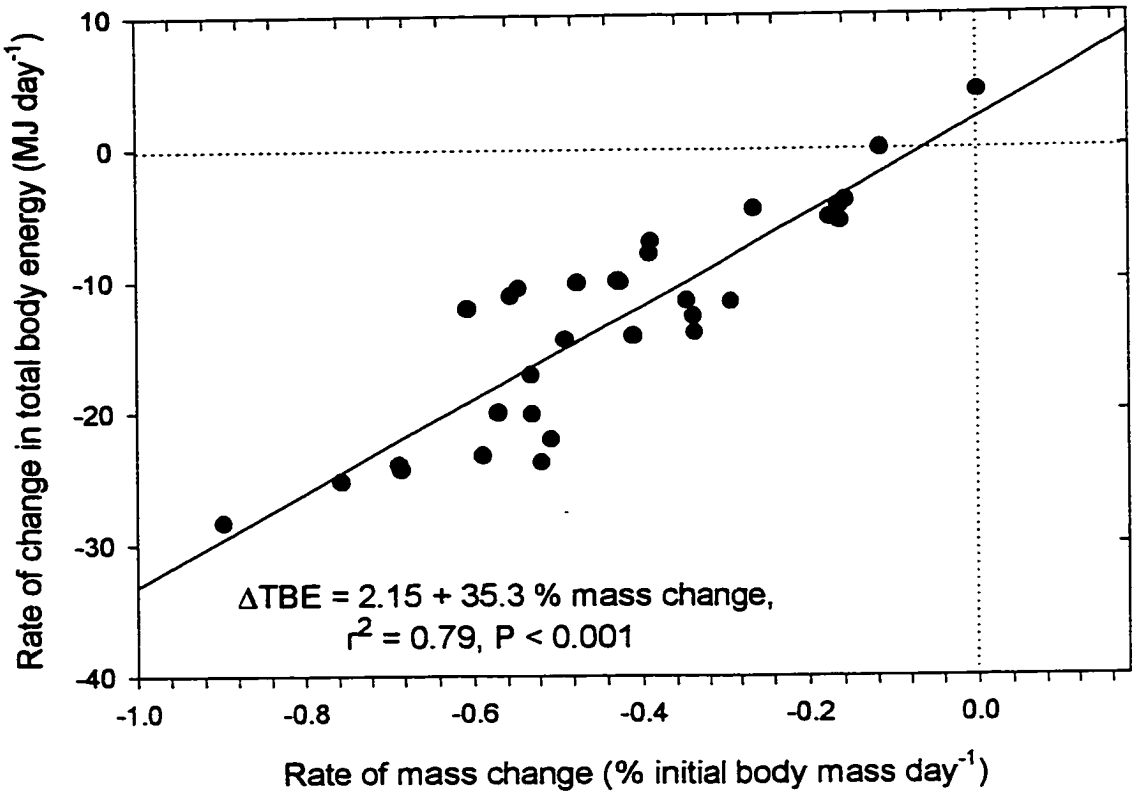


Figure 3.4. Rate of change of total body energy as a function of mass loss.

Table 3.2. Body water flux and energy expenditure of male harbour seals during the breeding season.

Parameter	n	Mean	Range	CV
Total water flux ($L\ day^{-1}$)	17	4.3 ± 0.2	3.0 to 6.1	20.3 %
Water intake from feeding ($L\ day^{-1}$)	17	3.4 ± 0.2	2.1 to 5.2	25.7 %
Metabolizable energy from feeding ($MJ\ day^{-1}$)	17	18.6 ± 1.2	11.5 to 28.1	25.6 %
Rate of change in body energy ($MJ\ day^{-1}$)	31	-13.1 ± 1.5	-28.3 to 4.2	61.9 %
Total energy expenditure ($MJ\ day^{-1}$)*	17	32.1 ± 2.1	17.7 to 52.0	27.5 %
Percent of energy from feeding	17	60.4 ± 3.9	34.4 to 99.7	26.6 %

* Total energy does not equal the sum of energy from feeding and change in total body energy shown in this table because the average change in total body energy shown here was estimated from a larger sample of males.

There were clear differences in the rates of mass change, ΔTBE, water flux, FEI and TEE between the pre-mating and mating periods of the breeding season (Table 3.3). Males lost on average 5.3 times as much mass and TBE per day during the mating period than they did in the pre-mating period. TWF was significantly higher during the pre-mating period than during the mating period, which suggests that they were feeding more intensely before most females became sexually receptive. We estimate that the metabolizable energy available to males from food intake (FEI) accounted for 92.4% of the TEE during the pre-mating period, whereas it only accounted for 36.2 % of TEE during the mating period. Mean TEE was higher during the mating period, but the difference was not quite statistically significant (P = 0.056, Table 3.3). On average, males also spent more than twice as much time in deep dives (15.6 minutes per hour) during the pre-mating period than in the mating period (6.4 minutes per hour), which is consistent with a reduction in time spent foraging in deeper water. Conversely, males spent significantly more time making dives to depths of less than 20m during the mating period than during the pre-mating period.

During the pre-mating period, FEI was significantly positively correlated with time spent deep diving (Figure 3.5) but not with shallow dive effort (following arcsine square-root transformation of the diving variables, r = 0.57 and -0.02, P = 0.034 and 0.94, respectively). During the pre-mating period, 31% of the variation in FEI among male harbour seals can be attributed to variation in the time they spent diving deeply (Figure 3.5), however, during the mating period,

Table 3.3. Comparison of water flux, energy balance and diving behaviour of male harbour seals during the pre-oestrus and oestrus periods of the breeding season.

Parameter	Pre-mating	Mating	t*	n	P
Mass change (kg day^{-1})	-0.15 ± 0.02	-0.88 ± 0.02	5.53	23	<0.001
Change in total body energy (ΔTBE) (MJ day^{-1})	-4.7 ± 2.4	24.7 ± 2.8	4.97	23	<0.001
Total water flux (TWF) (L day^{-1})	5.3 ± 0.4	3.6 ± 0.3	3.21	17	0.003
Energy from food (FEI) (MJ day^{-1})	23.8 ± 2.3	14.9 ± 1.6	3.12	17	0.007
Percent of total energy from FEI (%)	78.3 ± 5.6	42.5 ± 5.8	4.06	17	<0.001
Total energy expended (TEE) (MJ day^{-1})	29.1 ± 2.6	38.5 ± 2.7	2.53	17	0.022
Deep dive effort** (minutes > 20m per hour)	15.6 ± 2.0	6.4 ± 1.6	2.93	14	0.010
shallow dive effort** (minutes at < 20m per hour)	13.6 ± 1.9	21.0 ± 3.5	2.15	14	0.046

*- paired t-test
**- data from Chapter II

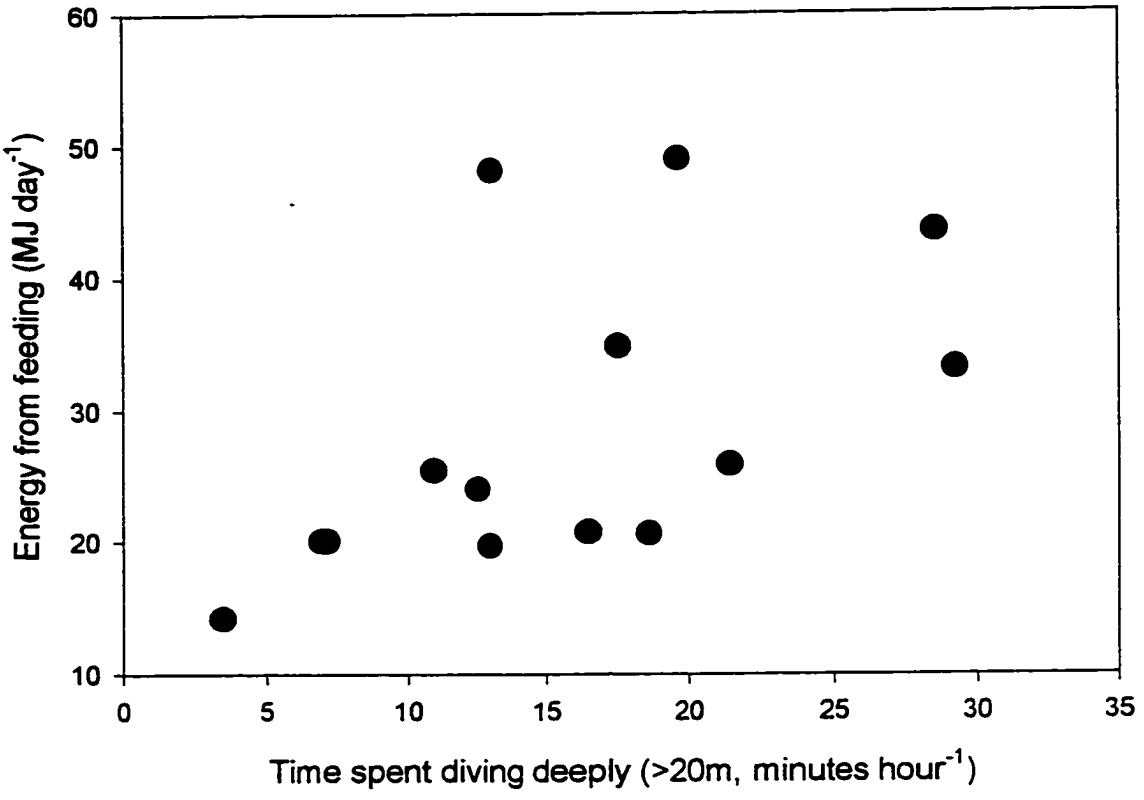


Figure 3.5. Rate food energy intake versus time spent deep diving during the pre-mating period. n = 17.

FEI was not significantly correlated with either measure of diving activity (r = 0.144 and -0.01 for deep and shallow dive effort, respectively).

Relatively large males, with initial mass greater than the median (108 kg), had similar rates of FEI as smaller males during the pre-mating and mating periods (one-factor repeated measures ANCOVA, initial mass included as covariate, F_{size} = 0.01; df = 1,14; P = 0.991) but lost significantly more TBE (one-factor repeated measures ANCOVA, initial mass included as covariate, F_{size} = 4.65; df = 1,20; P = 0.043) particularly during the pre-mating period (Figure 3.6). Both large and small seals had lower FEI and higher rates of ΔTBE during the mating period than during the pre-mating period (two-factor repeated measures ANCOVA F_{time} = 9.14 and 22.79; df = 1,15 and 1,21; P < 0.01 for each, respectively). Overall, relatively large males had higher TEE than smaller males over the entire breeding season (one-factor repeated measures ANCOVA, initial mass included as covariate, F_{size} = 6.78; df = 1,14; P = 0.021).

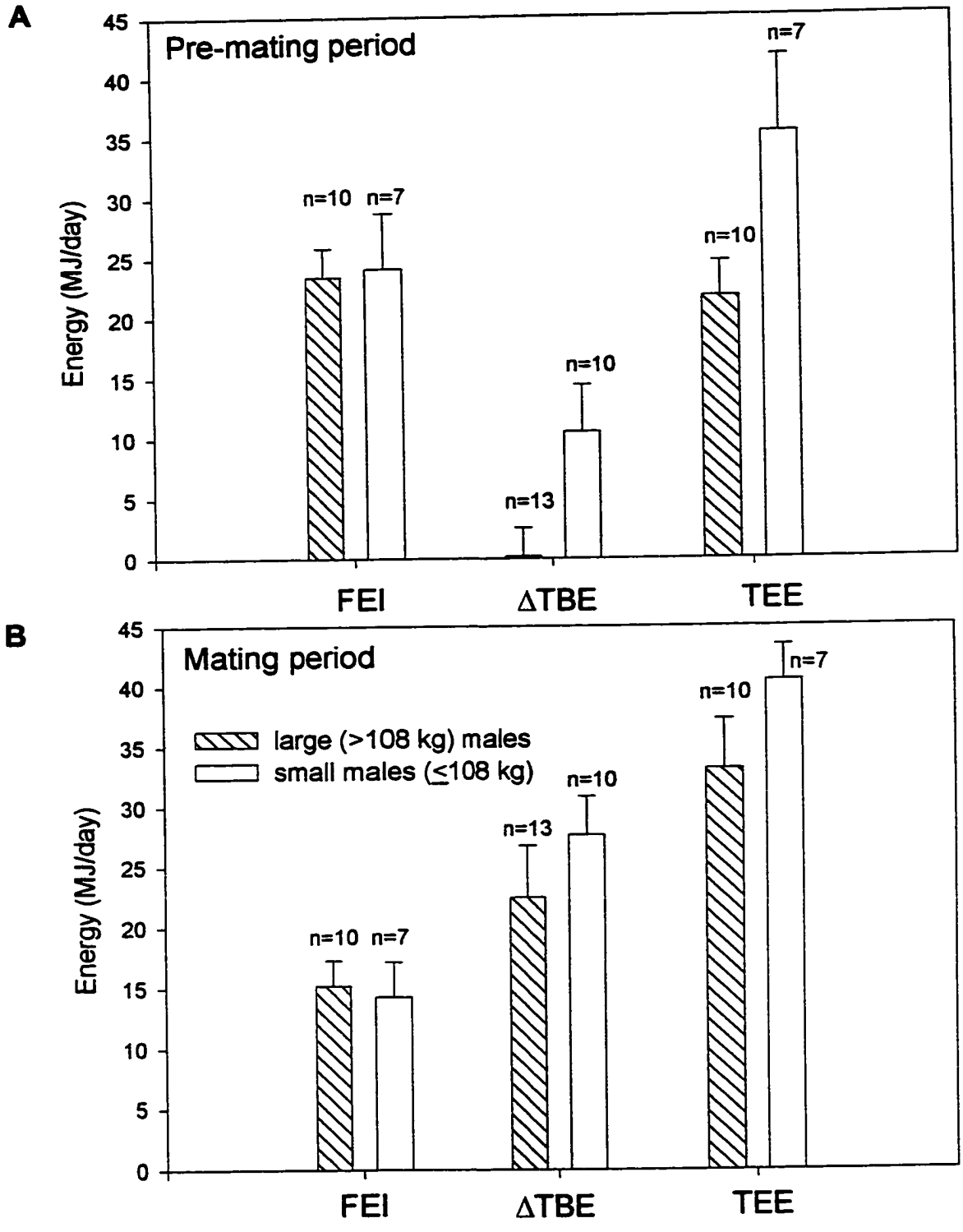


Figure 3.6. Rates of food energy intake, change in total body energy and total energy expended of male harbour seals. Data is shown for the pre-mating (A) and mating (B) periods of the breeding season.

DISCUSSION

Sources of error

Hydrogen isotope dilution methods of estimating TBW and body composition have been verified by carcass analysis for adults of two pinniped species, grey seals (Reilly and Fedak 1990) and Antarctic fur seals (Arnould **et al.** 1996), and from birth to weaning for ringed seal pups (Lydersen **et al.** 1992) and hooded seal pups (Oftedal **et al.** 1993). Inter-specific extrapolation of the relationships between body composition and TBW, however, may reduce their predictive accuracy (Arnould **et al.** 1996). Application to my data of the more widely used method of body composition estimation derived by Pace and Rathbun (1945), assuming the fat-free component of total body mass to consist of 73% water, gave very similar results to those from Reilly and Fedak's (1990) predictive equations (23.6 $\pm$ 0.6% versus 24.0 $\pm$ 0.6%, n = 35, t = 0.21, P = 0.83). Predictive equations derived by Arnould **et al.** (1996) for Antarctic fur seals gave quite different results; 21.1 $\pm$ 0.5 % initial body fat (t = 3.46, P = 0.001). I used the predictive equations derived by Reilly and Fedak (1990) for grey seals because they were empirically derived from a more closely-related phocid seal species, and because the range of body sizes included in their analyses spanned the range of my study animals.

Another source of error in this study may be the estimate of MWP from FMR. I derived the estimate of FMR from the relationship between ΔTBE and the rate of mass loss, made under the assumption that a fasting harbour seal loses 1.0% of it's initial body mass per day. Estimated this way, FMR (0.90 kg H_2O/day

generated from 33.2 MJ/day) is very close to the final estimate of TEE (32.1 MJ/day), and for an average sized male harbour seal (107.5 kg) it is 3.4 times the basal metabolic rate predicted by the equation of Kleiber (1975) for a similarly sized mammal. Deviation from this estimate of MWP by as much as plus or minus 50% resulted in small differences in the final estimates of TEE (i.e. if MWP = 0.45, TEE would = 33.4 ± 2.1 MJ/day; if MWP = 1.35, TEE would = 29.6 ± 2.1 MJ/day), so the estimate of TEE is robust to error in the assumed MWP.

Finally, it may appear that the assumption of a diet exclusively of sandlance would downwardly bias the estimate of FEI, since sandlance has a relatively low energy content (about 5 % fat). If I assumed a diet of higher energy content prey such as herring, which contains about twice as much fat as sandlance (68.2 % H_2O, 9.9 % fat, 17.5 % protein; Worthy 1990), the estimate of FEI would be only 5.9 % higher (19.7 vs. 18.6 MJ day^{-1}). The additional water produced by fat catabolism in a higher energy diet reduces the estimated quantity of food eaten, given the same rate of food water intake. Thus the estimate of FEI is fairly robust with respect to the exact composition of diet.

Changes in body mass and composition

These data show that adult male harbour seals on Sable Island have similar relative fat content (23.6%, n = 35) at the beginning of the breeding season as do territorial male Antarctic fur seals (24%, n=4) (Boyd and Duck 1991), and British harbour seals (20.1%, n=3) calculated from data in Reilly and

Fedak (1991). Male harbour seals lose mass and expend energy from initial body stores at a similar rate as do male northern elephant seals, but at approximately one half the rates estimated for males of other species of pinnipeds for which there are longitudinal data available (Table 3.4). However, when energy intake from feeding is added, I estimate that male harbour seals expend total energy at a similar rate as do terrestrially breeding grey and Antarctic fur seals and aquatically mating Weddell seals (Table 3.4). Estimates of ΔTBE from mass loss suggest that northern elephant seals expend energy at about half the rate of other breeding pinniped males. However, their breeding season is considerably longer than those of the other species shown in Table 3.4, and most dominant bulls remain on the rookery for the full duration of the breeding season (Deutsch **et al.** 1990). Given such a long period of tenure, male northern elephant seals bulls may minimize their rate of energy expenditure by spending most of their time inactive or sleeping. Furthermore, northern elephant seals are approximately an order of magnitude larger than males of the other species shown in Table 3.4; hence their mass-specific metabolic rate should be lower (Kleiber 1975, Lavigne **et al.** 1986, Nagy 1987).

The slope of the regression of the log of the estimated energy expenditure versus log body mass of the breeding male pinnipeds shown in Table 3.4 (0.78 ± 0.04) is not significantly different from 0.75 (Figure 3.7) and the rates of energy expenditure of male breeding pinnipeds shown in Table 3.4 are not significantly different ($t = 2.61$, $n = 5$, $P > 0.05$) from three times the basal metabolic rate predicted by Kleiber's equation (1975). This indicates that males of smaller-

TABLE 3.4. Body size, composition, and energy expenditure of breeding male pinnipeds.

	Harbour seal[1]	Weddell seal[2]	Grey seal[3]	Antarctic fur seal[4]	Northern elephant seal[5]
Mating environment	aquatic	aquatic	terrestrial	terrestrial	terrestrial
Duration of mating season	4 weeks	4 weeks	4 weeks	4 weeks	8 weeks
Initial body size (kg)	107.5	372	257	188	1704
Initial percent fat	23.6	na	na	24	na
Rate of mass loss (% initial mass day^{-1})	0.41	0.81	0.86	0.81	0.42
Rate energy expended from body stores (MJ kg^{-1} day^{-1})	0.12	0.30*	0.32*	0.30	0.16*
Total energy expenditure (W kg^{-1})	3.1	3.5**	3.7	3.2	1.8
Total mass loss (% of initial mass)	17***	30.0	17	24	36
Method and sample size for metabolic estimate	D_2O dilution n = 17	mass change n = 7	mass change, n = 33	doubly labelled water, n = 1	mass change, n = 17

1. this study.
2. Bartsh **et al.** (1992).
3. Anderson and Fedak (1985).
4. Boyd and Duck (1991).
5. Deutsch **et al.** (1990)

Other notes: na- data not available, *- calculated assuming density of mass loss = 36.7 MJ/kg, **- calculated assuming no energy intake from feeding, ***calculated from 1994 data only.

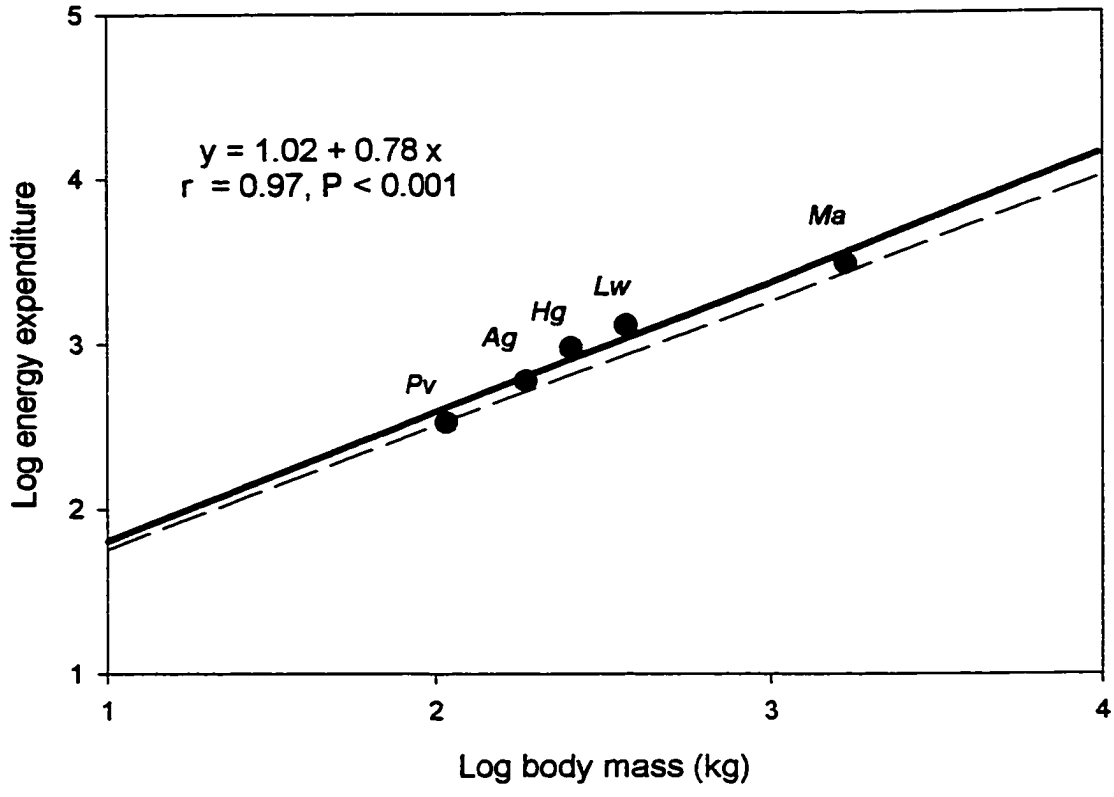


Figure 3.7. Rates of energy expenditure of breeding male pinnipeds. The dashed line represents three times the basal metabolic rate predicted by the equation of Kleiber (1975). *Pv*- harbour seal (this study), *Ag*- Antarctic fur seal (Boyd and Duck 1991), *Hg*- grey seal (Anderson and Fedak 1985), *Lw*- Weddell seal (Bartsh **et al.** 1992), *Ma*- northern elephant seal (Deutsch **et al.** 1990).

bodied pinniped species, such as the harbour seal, have less fasting endurance than males of larger species.

The energetic cost of reproduction for aquatically-copulating male harbour seals in our study is similar to that in species in which males compete for mates on land. Based on the measurement of metabolic rate in one animal, and the observation of higher relative rates of water flux for male harbour seals than for male grey seals, Reilly and Fedak (1991) suggested that male harbour seals expend energy at a much greater rate, the equivalent of six times the basal rate predicted by Kleiber (1975), than terrestrially mating pinnipeds. They further suggest that this high rate of energy expenditure was due to the high cost of aquatic displays and aggressive encounters with other males at sea. Our data suggest that a higher rate of water flux in male harbour seals can largely be attributed to water intake from feeding during the breeding season. While Reilly and Fedak's (1991) estimate of TEE from doubly-labelled water (52.5 MJ/day) was significantly higher than ours (32.1 $\pm$ 2.1 MJ/day; one-sample t-test; t=9.6; $P<0.001$, n=17), and falls just outside of the range of our estimates (17.7 to 52.0 MJ/day), it was based on a single observation. Male harbour seals probably do spend less time resting during the breeding season than male grey seals. Boness (1984) reported that male grey seals spend 88.8% of their time resting on shore whereas data from TDRs indicate that male harbour seals only spend 22.3% of their time hauled out (Chapter II), although this may underestimate the total time harbour seals spend resting because they spend some time sleeping while at sea (Boness, Bowen, Marshall and Buhleier, unpublished data).

Terrestrially breeding Antarctic fur seals and northern elephant seals also spend more than 50% of their time on shore resting during the breeding season (Boyd and Duck 1991, Deutsch **et al.** 1990). Since pinnipeds are morphologically and physiologically adapted to life at sea, it seems unlikely that aquatic courtship and mate competition is more energetically demanding than terrestrial mating. In fact, terrestrial breeding activity may be so expensive for phocid seals that males which mate on shore may spend most of their time resting to sustain tenure for as long as possible. Male harbour seals may be more active during the breeding season but aquatic activity is probably less energetically expensive per unit time than terrestrial activity for male pinnipeds.

Male Weddell seals also mate aquatically, but lose mass at a rate similar to territorially copulating species (Table 3.4). This suggests either that they do not feed during the breeding season, or that aquatic mating activity is very expensive for males of this species. Mass loss data alone would suggest a similar mass-specific rate of energy expenditure for breeding male Weddell seals as for males of other pinniped species (Table 3.4). Unlike male harbour seals, data from radio transmitters show that, like terrestrially mating pinnipeds, male Weddell seals spend most (52.4%) of their time resting on ice during the breeding season (Bartsh **et al.** 1992). One explanation may be that the local availability of prey may not make foraging worthwhile feasible for breeding male Weddell seals. The mating system of the Weddell seal is also probably atypical among aquatically mating species. Males compete for clearly defined local resources, leads in the fast ice which limit the access of males and females to

water, during the breeding season (Hill 1987). This may limit the extent to which territorial male Weddell seals can supplement their energy reserves by opportunistic foraging during the breeding season. The Weddell seal is also a relatively large bodied species, therefore it is less constrained by limited body stores than are males of smaller bodied aquatically mating species, such as the harbour seal.

Male body size, feeding and reproductive effort

Studies have previously demonstrated that male harbour seals feed early during the breeding season, and have suggested that the small body size of this species may have played a role in shaping male reproductive strategies (Thompson **et al.** 1989, Walker and Bowen 1993a, Chapter II). On Sable Island males tend to maintain their body mass during periods of deep diving prior to the time when females enter oestrus (Chapter II). These data indicate that on average 78% of the total energy expended by males prior to oestrus came from food intake (Table 3.3). In 7 of the 17 males, food intake equalled or exceeded TEE during the pre-mating period. All seven of these individuals actually gained mass during the pre-mating period. During the mating period, energy intake from feeding still accounted for about 42% of the gross energy expended, but there was great variation among males (range from 5.4 to 99.2 % of TEE, CV = 29.6%). The male in which only 5.4% of TEE was fueled by feeding had the second highest rate of mass loss (1.27 kg/per day) and ΔTBE (41.9 MJ per day) among the 17 water flux study animals, and it was the heaviest male in the study

(131.5 kg). The individual with the highest relative rate of FEI (99.2% of TEE) lost only 3 kg during the mating period, and had the least ΔTBE (less than 1 MJ per day) over the 25 day study period. Over the entire breeding season, energy intake from feeding contributed just over half of the total energy expended on average (60.4 ± 3.9%). If males were to rely entirely on their initial energy stores and fast throughout the breeding season, yet maintain an average rate of total energy expenditure of 32.1 MJ/day, an average male harbour seal (with 1500 MJ of TBE and 23.6% fat at the beginning of the breeding season) would deplete 50% of total body energy in about 3 weeks. Since most of this energy comes from fat catabolism, an average male would fall below 5% total body fat during the breeding season. Male harbour seals may feed since it is unlikely that they can afford to fast throughout the breeding season, yet maintain their levels of activity. Alternatively, if there is no clear advantage for a male harbour seal to establish a position at sea relatively early in the breeding season, it may not be necessary for males to fast throughout the breeding season.

As only about 30 % of the variation in FEI, or the rate of expenditure of TBE, can be attributed to variation in deep dive effort (Figures 3.3 and 3.5), male harbour seals may also be feeding during periods of shallow diving activity, or my measure of foraging effort, based solely on dive depth, may not be very accurate. Furthermore, these data indicate that male harbour seals derive more than one-third of their energy from feeding during the mating period, yet at the same time most males stopped making trips consisting of deeper dives (Chapter II). Opportunistic feeding, occurring within bouts of shallow display dives or while

patrolling home ranges near the shore, may therefore provide an important source of energy to maintain their reproductive effort

I previously hypothesized that larger males may have a mating advantage since they spend less time foraging and more time making shallow dives which are more likely to be associated with aggressive behaviours and displays associated with acquiring mates (Chapter II). Data presented in this study is not inconsistent with this hypothesis. Larger seals depleted their TBE during the pre-mating period (Figure 3.6) to a significantly greater extent than smaller males, and they spent significantly more total energy during the breeding season. Males which are relatively large at the beginning of the breeding season would appear to invest more energy in reproduction, at a cost in terms of depleting their TBE, which should translate into a mating advantage. Reproductive effort is a strong predictor of dominance status and mating success in other pinniped species such as grey seals (Anderson and Fedak 1985, Tinker **et al**. 1995) and northern elephant seals (Deutsch **et al**. 1990).

Harbour seals have limited size dimorphism; on Sable Island, adult males are approximately 6% longer (McLaren 1993) and 18% heavier than postpartum females (Bowen, Boness and Iverson, unpublished data). This would suggest that male body size may be less strongly selected for in the harbour seal than it may be in more dimorphic, terrestrially breeding pinniped species. Since mating and feeding both occur at sea in aquatically mating pinnipeds, relatively smaller males, or males in inferior condition at the beginning of the breeding season, may be at less of a disadvantage since they can feed during the mating season.

Larger males make more shallow dives earlier in the season, at a cost in terms of energy expended from TBE (Figure 3.6), which may provide them with a slight mating advantage if increased shallow diving activity improves their encounter rates with potentially receptive females. Males with large initial mass may also represent relatively older animals, or males which are in better overall condition. The latter may be less likely, however, as large and small males had similar body composition (Figure 3.2). Differences between large and small males may therefore also reflect the effects of previous breeding experience in addition to general body size and TBE.

For male harbour seals, and perhaps other pinnipeds which mate at sea, the classic paradigm of the complete temporal and spatial separation of feeding and reproduction acting as a dominant factor in the evolution of pinniped mating systems and size dimorphism (Bartholomew 1970) may be less applicable than it is for terrestrially mating species. The reproductive strategies, mating systems, and the evolution of size dimorphism in aquatically mating pinnipeds has been subject to a different set of ecological variables (such as the local distribution of prey in the mating environment) which are likely to vary between species and populations. For male harbour seals on Sable Island, relatively large size may not greatly enhance mating success if foraging can supplement initial energy reserves to cover the energetic costs of reproduction. Smaller males may improve their mating success by feeding more until the time when most females are receptive. For aquatically mating pinnipeds, male reproductive strategies may be influenced by the local distribution of consumable resources may as well

as by phylogenetic and ecological factors that were critical in the evolution of mating systems by determining the availability and the economic defensibility of oestrus females in time and space (Emlen and Oring 1977, Stirling 1983, Boness 1991).

CHAPTER IV

PATERNITY AND MATING SUCCESS OF MALE HARBOUR SEALS DETERMINED BY MICROSATELLITE DNA FINGERPRINTING

INTRODUCTION

Studies of male mating success in pinnipeds have mostly been limited to terrestrially breeding species through observing associations between individuals during the breeding season (Boness **et al.** 1993). In these species, estimates of the level of polygyny, or the variance in mating success among males, have been made from observed copulations between marked individuals (Boness and James 1979; Le Boeuf 1974) or from the ratio of the number of reproductive females to the number of reproductive males (Boyd 1989; see references in Boness **et al.** 1993). Generally, in terrestrially mating species receptive females are predictably clustered in space and time. Studies have shown that males which can successfully defend resources that attract females, such as beach space within a colony (territory or resource defence polygyny), or aggregations of females themselves (harem or female defence polygyny) may realize exceptional mating success (e.g. northern fur seals, *Callorhinus ursinus*: Bartholomew and Hoel 1953; northern elephant seals, *Mirounga angustirostris*- Le Boeuf 1974). As a result, the variance in male mating success in terrestrially mating species is thought to be high, and these species are generally considered to be polygynous (Stirling 1983; Boness 1991; Boness **et al.** 1993).

Two otariid species and fifteen species of phocid seal, representing almost all the Phocidae, mate aquatically, and very few studies have attempted to measure male mating success due the inherent difficulties in observing behaviour which occurs only at sea (Le Boeuf 1991; Boness 1991; Boness **et al.** 1993). It is generally thought that the variance in male mating success should be lower when copulation and courtship occur in the water since males are less able to control the access of receptive females to other mates in a three-dimensional environment, and females are considerably more mobile in the water than on land (Bartholomew 1970; Stirling 1983).

Among the aquatically mating pinnipeds, the mating systems of the Weddell seal, which usually copulates in the water, and the Juan Fernandez fur seal, which predominately mate on shore but also mate aquatically, are perhaps the most well-characterized. Using the transfer of coloured grease from marked males to females to infer copulations, Hill (1987) and Bartsh **et al.** (1992) found that a group of male Weddell seals may have monopolized matings to a limited extent by defending aquatic territories around leads in the fast ice which females must use for access to the sea (a form of resource defence polygyny). Juan Fernandez fur seals usually mate on shore, but a small number of males which maintained aquatic territories in a lagoon used by females for daily thermoregulatory trips were observed to achieve as many copulations at sea as did dominant males which held terrestrial territories on the breeding beach (Francis and Boness 1991). Neither of these examples may be typical for aquatically copulating species; in both study populations there were clearly

defined local resources, important to breeding females, which a small number of males could control. When females are dispersed because critical resources such as beach space, access to water or a safe pupping site do not constrain their distribution, as it would appear to be for female hooded seals which breed on pack ice floes, males may attempt to guard and mate with relatively dispersed individual females sequentially (serial monogamy or sequential polygyny; Boness **et al.** 1988; Kovacs 1990). The variance in male mating success may be low under these circumstances because males cannot attend many females simultaneously, and they must spend time and energy searching for subsequent mating opportunities during a relatively short mating season.

While the distribution and mobility of females in the water may limit the extent to which males can control female movements and their access to other mates, aquatic mating may also give females the opportunity to exercise mate choice for the same reasons. Males may attempt to attract females to mating territories or home ranges by advertising through display behaviour if they cannot predict female locations or movements (Clutton-Brock 1989). When these mating territories are tightly clustered together or overlap in an arena (or lek), the variance in male mating success may be high if males vary widely in their quality or their ability to attract females, and in most lekking species there is pronounced sex dimorphism (Bradbury and Gibson 1983). Therefore, there is a possibility that females may be strongly biased in their choice of mates, which may lead to a high variance in male mating success in some aquatically mating species.

Molecular genetic methods offer the opportunity to address these issues in aquatically mating species, by analyzing paternity genetically. Major advances in the study of mating systems have been made possible by the development of a variety of genetic fingerprinting methods in the last decade (Burke 1989; Bruford and Wayne 1993), and some studies have clearly shown that estimates of mating success based on observed copulations may be inaccurate. In some cases, behavioural observations have underestimated mating success determined by genetic fingerprinting as in red deer, *Cervus elaphus* (Pemberton **et al.** 1992), while in others genetic evidence showed that observed copulations overestimated male mating success such as Japanese macaques, *Macaca fusculata*: (Inoue **et al.** 1991) and captive harbour seals (Harris **et al.** 1991).

The most commonly employed DNA-typing method of multilocus DNA fingerprinting (Jeffreys 1985ab) is accomplished by the simultaneous detection of alleles at multiple minisatellite loci using polycore minisatellite probes. Multilocus DNA fingerprinting provides accurate paternity results and can be applied to a wide range of organisms without the development of species-specific probes (Burke 1989). However, it requires relatively large amounts of high quality DNA for restriction digestion and Southern blotting, and it necessitates prior identification of the likely sires so they can be analyzed within the same gel making large population surveys logistically difficult (Tautz 1990; O'Reilly and Wright 1995). In multilocus DNA fingerprints, bands are sized relative to each other, and minor inconsistencies in running conditions between gels commonly result in band-shifting phenomena which makes the comparison

of patterns between gels problematic (Lewin 1989). Single-locus genetic markers, such as individual microsatellite loci, are therefore preferable to complex multilocus DNA fingerprints when the identity of the possible parents is unknown and comparisons are made between gels.

Microsatellites (Litt and Luty 1989; Weber and May 1989; Tautz 1989) are short stretches of simple DNA sequences (1 - 4 base pairs) arrayed in tandem which may be polymorphic in length due to slippage errors in DNA replication (Levinson and Gutman 1987; Tautz 1989). Since microsatellites are short (total length usually less than 200bp) and they are usually flanked by unique stretches of DNA, they can be efficiently amplified by the polymerase chain reaction (PCR-Saiki **et al.** 1988) and resolved to differences in length of a single base pair (bp) in polyacrylamide sequencing gels (Tautz 1990). Because of this, similarly sized alleles from individuals in separate gels cannot be confused as the alleles are sized absolutely rather than relative to bands in other lanes of the same gel. Additionally, if PCR primers are selected to ensure non-overlapping size ranges of products, products from several different loci can be run in the same gel with confidence that identically sized alleles in different individuals are from the same locus, unlike multilocus DNA fingerprints in which the source and allelic state of bands are unknown. Finally, reliable microsatellite amplification by PCR is possible from trace amounts of potentially degraded genetic material such as sloughed whale skin (Richard **et al.** 1996) or buccal cells in wadges (Takenaka **et al.** 1993b). Microsatellites markers therefore offer the desired characteristics for paternity analysis in any organism, but particularly for taxa in which there is

no prior identification of the paternal candidates because the data is amenable to storage and analysis between gels in a database (Tautz 1990; O'Reilly and Wright 1995). To date, microsatellites have been applied to studies of paternity in a relatively small, but rapidly increasing number of natural mammalian populations such as Japanese macaques (Takenaka **et al.** 1993a), chimpanzees, *Pan troglodytes*: (Morin **et al.** 1994) and grizzly bears, *Ursus arctos*: (Craighead **et al.** 1995) since PCR primers are relatively specific to the taxa in which they were originally developed.

I sought to determine the paternity of pups born in 1994 and 1995 on Sable Island using polymorphic microsatellite markers. In this chapter, I use this information to estimate the variance in mating success among male harbour seals in the Sable Island population, and to identify probable mate pairs to investigate overall mating patterns. Since no pinniped microsatellite sequences were available at the start of the study in 1992, I first developed primers for PCR amplification by cloning and sequencing harbour seal microsatellite DNA, then tested and employed pinniped microsatellite markers developed by other laboratories as they became available. The markers that detected the greatest levels of variability were used to determine patterns of paternity. I hypothesized that the variance in mating success among males would be relatively low compared to terrestrially mating pinniped species since male harbours seals may be less able to monopolise the access to, and movements of, receptive females in the aquatic environment. Furthermore, at Sable Island females have unlimited access to the sea and they are fairly widely distributed along the 25km

region of the north beach which is used for pupping, so it may be difficult for individual males to secure a large number of mates in a single season.

With the genetic paternity data, I also hoped to gain a better general understanding of the mating patterns in this population by comparing the locations of males to females with which they had mated. If males tend to mate with females which hauled out nearby, this would support the home range strategy suggested by Walker and Bowen (1993b). They suggested that males may attempt to establish an aquatic home range near their haul-out location and attempt to intercept and mate with females as they pass through their range during foraging trips in late lactation. If females mate with males without respect to their haul-out locations within the colony, attracting females to mating territories (either clustered in the form of a lek, or dispersed aquatic home ranges) or searching for receptive females are other possible male mating tactics. Data from other animals suggests that the variance in mating success among males in lekking species may be high (Clutton-Brock 1989), whereas the variance among males which search for mates ought to be low at Sable Island, where females are widely dispersed and their locations unpredictable. Secondly, if female choice is an important component of the mating system of harbours seals, and males vary greatly in their intrinsic quality or attractiveness to females, we might expect male mating success to be correlated between years, or for females to choose the same mate in subsequent seasons (mate fidelity) as has been suggested for grey seals at North Rona, Scotland by Amos **et al.** (1995). Since male and female harbour seals exhibit seasonal fidelity to Sable

Island, and some individuals even show fidelity to the same beach site between seasons (Schaeff **et al.,** unpublished data), the population is relatively small, and male harbour seals make individual-specific acoustic displays (Hanggi and Schusterman 1994), it may be possible for seals to recognize and select previous partners for mating. To test the mate fidelity idea, I measured the relatedness between pups born to the same female in different years in relation to the level of relatedness expected between half-sibs.

METHODS

Study site and sampling strategy

Sampling was conducted during the breeding seasons (mid-May to the end of June) of 1993, 1994 and 1995 on the north beach of Sable Island (43°55'N; 60°00'W), a partially vegetated sandbar 160 km east of Halifax, Nova Scotia. Between 200 and 400 harbour seal females gave birth on both the north and south beaches of Sable Island during each year of the study.

Skin samples were taken from males during behavioural studies in 1993, 1994 and 1995 for use in the paternity analysis. All of the sampled males were genetically compared to the 1994 and 1995 pup cohorts born within the 25km study area of the north beach to identify the most-likely sires.

Sample collection and template DNA extraction

During routine tagging of all pups born on the north beach of Sable Island in 1994 and 1995, a 6 mm punch was used to create a hole in the webbing of the hindflipper prior to insertion of an individual-specific permanent tag (Dalton Industries). The biopsied skin and underlying tissue was stored in a 5 ml plastic tube containing a preservative solution of saturated NaCl / 20% w/v dimethyl sulfoxide. We attempted to capture mothers simultaneously when handling pups for tagging. A skin sample was similarly taken from the mother's hindflipper and from every male handled and tagged during other behavioural and energetic studies.

Skin samples were stored at 4° C or -20° C from the day of collection until extraction. Template DNA was extracted from skin by incubating 5 mg in 200 µl of 5% Instagene DNA Purification Matrix (Bio-Rad) overnight at 60° C in a 1.5 ml microfuge tube, then immersing the tube in a boiling water bath for 8 minutes. Two microliters of the supernatant were routinely used as the template for PCR amplification.

Development of PCR primers for harbour seal microsatellite loci

High molecular weight genomic DNA of a single harbour seal was extracted from blood by standard methods (Sambrook **et al.** 1989) and digested with a mixture of the restriction enzymes *Alu* I, *Hinc* II and *Hae* III (Pharmacia). Fragments were separated in a 1% low melting point agarose gel and DNA from a gel slice containing the 300-800bp size fraction was extracted by standard techniques (Sambrook **et al.** 1989). DNA was ligated into *Sma* I digested dephosphorylated pUC18 (Pharmacia). "Max-efficiency" DH5α cells (BRL) were transformed, plated on selective media and colonies were immobilized on Amersham N+ nylon membranes. Membranes were pre-hybridized for 3 hours in 5 x SSPE / 0.1% SDS / 5 x Denhardt's / 10 mg/mL RNA at 58°C. For a probe, 100 ng of $(GT)_{15}$ oligonucleotide was end-labelled with T4 polynucleotide kinase (Pharmacia) and γ-[^{32}P]-dATP to high specific activity. The probe was added to the mixture and hybridization was allowed to proceed overnight at 58°C. Membranes were washed twice at room temperature and once at 45°C in 2 x SSPE / 0.1% SDS for 15 minutes and exposed to X-ray film overnight at -70°C.

Positively hybridizing colonies were sampled, cultured, then plasmid DNA was extracted for sequencing following the protocol of Goode and Feinstein (1992). DNA sequencing was performed using T7 DNA polymerase (Pharmacia) and forward and reverse universal primers.

For each suitable locus, primers were designed to have similar annealing temperatures and less than 50% self and inter-complementarity. For PCR amplification, one primer from each pair was end-labelled with T4 polynucleotide kinase and [γ^{32}P]-ATP. PCR was carried out in a 5 μl cocktail consisting of 2μl template DNA, 0.2 μM of each primer, 200 μM each dNTP, 10 mM Tris-HCl, 50 mM KCl, 2 mM $MgCl_2$ and 0.001% gelatin with 0.5 units of *Taq* polymerase. PCR amplification was performed in a PTC-100 thermal cycler (MJ Research) and consisted of 7 cycles of 30s denaturation at 94°C, annealing at primer-specific temperature, and elongation at 72°C; followed by 28 cycles of 30s denaturation at 89°C, annealing at 2° above the primer-specific temperature, and elongation at 72°C. PCR products were separated by electrophoresis in standard denaturing polyacrylamide gels and visualized by autoradiography. I also tested these primers for amplification of microsatellites in other phocid and otariid seal species.

Assessment of polymorphism and selection of loci for paternity analysis

In addition to the markers I developed, the degree of polymorphism at other microsatellite loci developed for pinnipeds by S. Goodman *(Phoca vitulina vitulina*, n=7) and P. Allen (*Halichoerus grypus*, n=8) in the Department of Genetics at Cambridge University (Cambridge, UK), and R. Slade *(Mirounga leonina*, n=1) at the

University of Queensland, were initially assessed by amplifying samples from Sable Island harbour seals. All loci which reliably amplified products of the expected size range and had at least 3 distinct alleles in the initial screening were employed in the analysis of paternity.

Genetic data collection and paternity analysis

Following electrophoresis and autoradiography, allele sizes were estimated by comparing the migration distance of bands to a single base pair ladder generated by sequencing bacteriophage M-13 DNA. Allele frequencies were calculated from the observed adult genotypes in each year of sampling. Observed genotype frequencies were tested for homogeneity between years and deviation from Hardy-Weinberg equilibrium (HWE) by χ^2 goodness-of-fit tests.

The probability of 2 unrelated individuals sharing the same genotype at a given locus (probability of identity at locus *k*, $P(id)_k$) was calculated as:

$$P(id)_k = \Sigma_i (f(g_i))^2$$

where $f(g_i)$ represents the frequency of the *i*th genotype at locus *k*. The cumulative $P(id)_c$ across all *l* loci was then estimated as:

$$P(id)_c = 1 - \Pi_l (1-P(id)_k)$$

For each *k* locus in each year, the average probability of excluding a randomly chosen male as a possible genetic sire of a pup ($P(ex)_k$) was estimated as:

$$P(ex)_k = {}_{j=1}\Sigma_n \, (\Sigma \, f(g_{exj})) \, / \, n$$

where $f(g_{exj})$ equals the frequency of paternal genotypes which could not have supplied the paternal allele for each *j* pup, estimated from population allele frequencies, and n represents the total number of pups sampled in that year. The frequency of paternal exclusions observed for each locus *k* ($f(ex)_k$) and overall ($f(ex)_c$) were similarly calculated as:

f(ex) = # of exclusions observed / # of pair-wise comparisons

where an exclusion is here defined as the absence of a paternally diagnostic alllele for a given pup in a particular male's genotype.

I used formulae derived by Thompson (1976) to calculate log-likelihood ratios for paternity analysis. For each pup, the paternal contribution at each locus was first determined by subtracting the maternal contribution to the pups genotype. A matrix of all possible pair-wise comparisons of male genotypes and paternally derived alleles was then created for each locus, and the probability of male *m* producing the paternal genetic contribution (g_x) across all *l* loci was calculated as:

$$P\ (g_x|G_m) = \prod_l T_k\ (A_p|G_m)$$

where G_m represents the genotype of the *m*th male, and $T_k\ (A_p|G_m)$ represents the Mendelian transition probability of the *m*th male providing the paternal allele (A_p) at the *k*th locus multiplied across all *l* loci used in the paternity analysis. The paternity index, or the odds of paternity vs. non-paternity, for each comparison was calculated as the ratio of the transition probability of the mth male ($g_x|G_m$) to the probability of sampling g_x from a randomly selected male ($P\ (g_x|G_r)$):

$$Odds_m = P\ (g_x|G_m)\ /\ P\ (g_x|G_r)$$

$$= \prod_l T_k\ (A_p|G_m)\ /\ \prod_l P_k\ (A_p)$$

where $P_k\ (A_p)$ represents the probability of sampling allele *p* at locus *k*. Assuming unlinked loci and HWE, this value was taken as the frequency of the *p*th allele at locus *k*. If 2 alleles were equally likely (the maternal and pup genotypes were heterozygous for the same alleles) $P_k\ (A_p)$ was taken as the mean frequency of the 2 alleles. The natural logarithm of the likelihood ratio (LOD score, or log-likelihood) was calculated for each male with $P\ (g_x|G_m)$ greater than zero, and the male with the highest LOD was considered to be the most-likely sire. Inferences about paternity were made according to the magnitude of the

difference between the LOD of the most-likely and the next most-likely sire (ΔLOD).

To my knowledge, there are no published criteria for accepting or rejecting ΔLOD scores to assign progeny to sires in paternity analysis in studies of natural populations. Previous studies have assigned progeny to the most-likely male candidate (Meagher 1986) or to unique non-excluded males (Morin **et al.** 1994). Likelihood ratios of 20:1 (LOD = 3.00) are generally considered sufficient for human paternity tests conducted by commercial laboratories (Genetic Design Inc, USA). I used a program created by Tristan Marshall (PATSIM version 1.1, University of Edinburgh, Edinburgh UK) to calculate critical ΔLOD scores to accept the hypothesis of paternity vs. non-paternity for a given level of confidence (1-α) using a Monte Carlo algorithm based on the allele frequencies of the loci used. A detailed description of the program can be obtained at http://www.ed.ac.uk/~tcm. The input parameters for the program, and the values I used to generate a distribution of critical ΔLOD scores versus the user-specified probability of false inclusion (α) are as follows:

1) Number of randomized cycles-	10 000
2) Total number of candidate males-	200
3) Proportion of candidate males sampled-	0.45
4) Proportion of genotypes scored	1.00
5) Proportion of mis-scored genotypes	0.01
6) Confidencece level (1-α)	0.35 - 0.95 (in 0.05 increments)

The total number of candidate males (parameter 2) was estimated from field observations with a Lincoln-Petersen index based on the frequency of sightings of marked males during periodic censuses of the total north beach population:

$$\frac{\text{Marked males sighted}}{\text{Total marked males in population}} = \frac{\text{Total males sighted}}{\text{Total \# of males in population}}$$

The mean estimate of the total number of males in the population was scaled to account for the presence of sub-adult males in the population which are unlikely to be reproductively active (Walker and Bowen 1993b) to estimate parameter 2. The proportion sampled (parameter 3) is the ratio of the total number of samples from males which were typed at all loci used in the paternity analysis to the total number of candidate males. Only males with complete genotypes were included in the paternity analysis (parameter 4 = 1.00). The error estimate (parameter 5) is used to correct for the possibility of false exclusion based on paternal mutations and the rate of observer error in scoring and recording allele sizes.

The confidence level (1-α, parameter 6) was interpreted as the probability of paternity of the most-likely sire. Probabilities of paternity for each most-likely sire on each pup were then estimated from the critical ΔLOD generated by PATSIM 1.1 (Results). The probability of mating success (here defined as the probability of siring at least 1 pup) for each *m*th male (P_{MSm}) was estimated as:

$$P_{MSm} = 1 - \prod_n \alpha_j$$

where α_j represents the probability of false inclusion for the *j*th pup for which male *m* was the most-likely candidate, multiplied across all *n* pups for which male *m* was the most-likely sire.

I also estimated the maximum number of pups a male may have sired (MS_{max}) as the sum of all pups for which a given male was genetically identified as the most-likely sire. Finally, the most-likely number of pups sired by a male *m* (MS_{ML_m}) was estimated as the maximum-likelihood solution of the function:

$$MS_{ML_m} = f\,(1-\alpha_j)$$

which defines the discrete probability distribution of siring 0 to *j* pups for which the *m*th male is the most-likely sire. The variances in MS_{ML} and MS_{max} among all sampled males were used as lower and upper estimates, respectively, of the variance in mating success among the sampled males in each year of the study.

RESULTS

Sample collection

Skin samples were collected from 91 males, 197 females and 275 pups between 1993 and 1995 (Table 4.1). A maternal sample was available for 260 pups. In 1994 the genotypes of the mothers of 2 pups were unknown, in one case because we did not capture the mother and the other because there was genetic evidence that the attending female was not the biological mother (see below). The identify of 13 mothers of pups born in 1995 was unknown (Table 4.1), and I found genetic evidence indicating that the attending female was not the biological parent in two other cases in 1995. I assumed that a male sampled in a given year was also present at Sable Island during previous and subsequent breeding seasons, therefore all males sampled between 1993 and 1995 were included in the paternity analysis in both years.

Development and polymorphism of harbour seal microsatellite loci

Approximately 10,000 recombinant clones were screened for (CA) microsatellites in 6 genomic libraries. One hundred and twenty-five clones which hybridized to the probe were sequenced in both directions. From preliminary DNA sequence analyses I found that approximately 40% of the microsatellite sequences I cloned were associated with another form of repetitive DNA, either short or long interspersed elements (SINEs and LINEs). These associations have been described

Table 4.1. Number of skin samples collected from harbour seals used in the analysis of paternity on Sable Island, Nova Scotia.

Type	1993	1994	1995	Total
Males	45	29	17	91
Females[1]	0	143	54	197
Pups[2]	0	144 (1)	131 (13)	275 (14)

1. The pups of 63 females previously sampled in 1994 were also sampled in 1995.
2. The number in parentheses refers to the number of pups for which there was no maternal sample available.

in more detail elsewhere (Coltman and Wright 1994; Duffy **et al.** 1996). Primers were not designed for the amplification of SINE or LINE associated microsatellites since there may be multiple copies of the flanking sequences in the seal genome which may prevent effective amplification of the locus.

For the remaining clones, 14 primer sets were designed and tested for amplification of harbour seal DNA. Eight primers amplified products of the expected size and, with the exception of *Pvc* 74, generally showed segregation between mothers and pups consistent with a pattern of Mendelian inheritance of codominant autosomal alleles. *Pvc* 74 appeared to be X-linked; among unrelated harbour seals, no heterozygous males were observed (n=25), whereas 10 of 25 females appeared to be heterozygous. Furthermore, all male offspring (n=10) tested possessed only one allele derived exclusively from the mother. Allelic variability and heterozygosity at each locus was initially investigated using tissue samples from approximately 50 presumed unrelated male and female adult individuals from Sable Island, Nova Scotia (Table 4.2).

Among phocid seals, cross-species amplification was also investigated using DNA from 10 grey seals (*Halichoerus grypus*), 2 hooded seals (*Cystophora cristata*) and 3 harp seal (*Phoca groenlandica*). Genomic DNA from representatives of three otariid species, the Antarctic fur seal (*Arctocephalus gazella*, n=5), the Subantarctic fur seal (*A. tropicalis*, n=3), and the New Zealand fur seal (*A. forsteri*, n=3) was generously provided by S. Goldsworthy (National Zoological Park, Smithsonian Institute, Washington D.C.). All of the loci tested were amplified by the harbour seal

Table 4.2. Number of alleles amplified by PCR primers developed for harbour seal microsatellite loci in other pinniped species.

Locus	Annealing temperature (°C)	Repeat structure[1]	Harbour seal (n=50)[2]	Grey seal (n=10)	Hooded seal (n=2)	Harp seal (n=3)	Antarctic fur seal (n=5)	New Zealand fur seal (n=3)	Subantarctic fur seal (n=3)	GenBank Accession Number
Pvc 19	53	$(CA)_{15}$	2 (0.50)[3]	2	4	4	4	4	3	L40983
Pvc 26	44	$(CA)_{9}$	1 (0.00)	1	3	1	1	2	1	L40984
Pvc 29	45	$(CA)_{7}$	2 (0.05)	1	1	1	5	6	5	L40985
Pvc 30	45	$(CA)_{13}$	2 (0.25)	2	3	5	2	2	3	L40986
Pvc 43	53	$(CA)_{15}$	4 (0.56)	nt	nt	nt	nt	nt	nt	
Pvc 63	45	$(CA)_{16}$	2 (0.07)	6	2	4	na	na	na	L40987
Pvc 74	45	$(CA)_{12}$	2 (0.40)	7	4	2	na	na	na	L40988
Pvc 78	52	$(CA)_{15}$	3 (0.16)	2	4	2	5	5	2	L40989

1. Number and sequence of largest uninterrupted array of repeats within target region for amplification.
2. n= number of samples amplified in the initial screening.
3. Observed frequency of heterozygotes, calculated for harbour seals only.

nt- Samples not tested for amplification.

na- Primers did not amplify a product.

primers in other phocid seals, while 5 were amplified in the otariids. These results are also partially described in Coltman **et al.** (1996).

PCR amplification of microsatellite loci

I tested primers for 16 other pinniped microsatellite loci developed in other laboratories on harbour seal samples. Four loci (PV 11 and PV 3: S.Goodman, unpublished data, Genbank accession numbers PVU6544 and PVU65442; H.g. 8.10: Allen **et al.** 1996; β–g: R. Slade, unpublished data) showed sufficient levels of variability to be useful for the paternity analysis, in addition to two of the microsatellite markers I developed (*Pvc* 19 and *Pvc* 43, see Table 4.2). I considered a locus with 2 or fewer alleles and heterozygosity of less than 0.4 not to be useful for paternity analysis. Observed heteozygosities ranged from 0.43 to 0.82 among the loci selected for use in the analysis of paternity (Table 4.3). P(id) ranged from 0.030 (PV 3) to 0.419 (*Pvc* 19) and generally varied inversely with the heterozygosity, number of alleles per locus and the evenness of the frequency distribution of allele sizes (Figure 4.1). Alleles were clearly not evenly distributed by size (Figure 4.1). Pvc 19 and PV11 (Figures 4.1A and 4.1E) showed one dominant allele size; Pvc 43, H.g. 8.10 and β-g had two relatively common allele sizes (Figures 4.1B, 4.1C and 4.1D, respectively) and PV 3 (Figure 4.1F) had several relatively common alleles and many rarer allele sizes. The cumulative P (id) across all 6 loci was 6.6×10^{-5}. Two males which were sampled in the same year shared the same genotype at all six loci, all other adult genotypes were unique.

Table 4.3. Polymorphism characteristics of microsatellite loci. Data are from all unique adult male and female samples used in the analysis of paternity in harbour seals on Sable Island, Nova Scotia.

Locus	Source[1]	Alleles	P (id)	H_o[2]	H_e[3]	n[4]	χ^2 (df)[5]	P
Pvc 19	a	2	0.419	0.46	0.43	285	0.70 (1)	0.71
Pvc 43	a	4	0.308	0.54	0.54	270	0.76 (3)	0.98
PV 3	b	21	0.030	0.82	0.88	271	5.48 (7)	0.60
PV 11	b	5	0.388	0.43	0.41	286	1.28 (6)	0.97
HG 8.10	c	5	0.340	0.58	0.52	286	1.45 (3)	0.69
β-g	d	5	0.129	0.73	0.71	284	1.05 (9)	1

1. Source of locus; a- this study, b- S. Goodman (unpublished), c- Allen **et al.** (1996), d- R. Slade (unpublished data).
2. Observed frequency of heterozygous individuals.
3. Expected frequency of heterozygous individuals assuming Hardy-Weinberg equilibrium.
4. Number of samples typed.
5. χ^2 goodness-of-fit test for deviation from expected genotype frequencies assuming Hardy-Weinberg equilibrium. Reduced degrees of freedom (df) reflect combined observations when expected cell size was less than 5 observations.

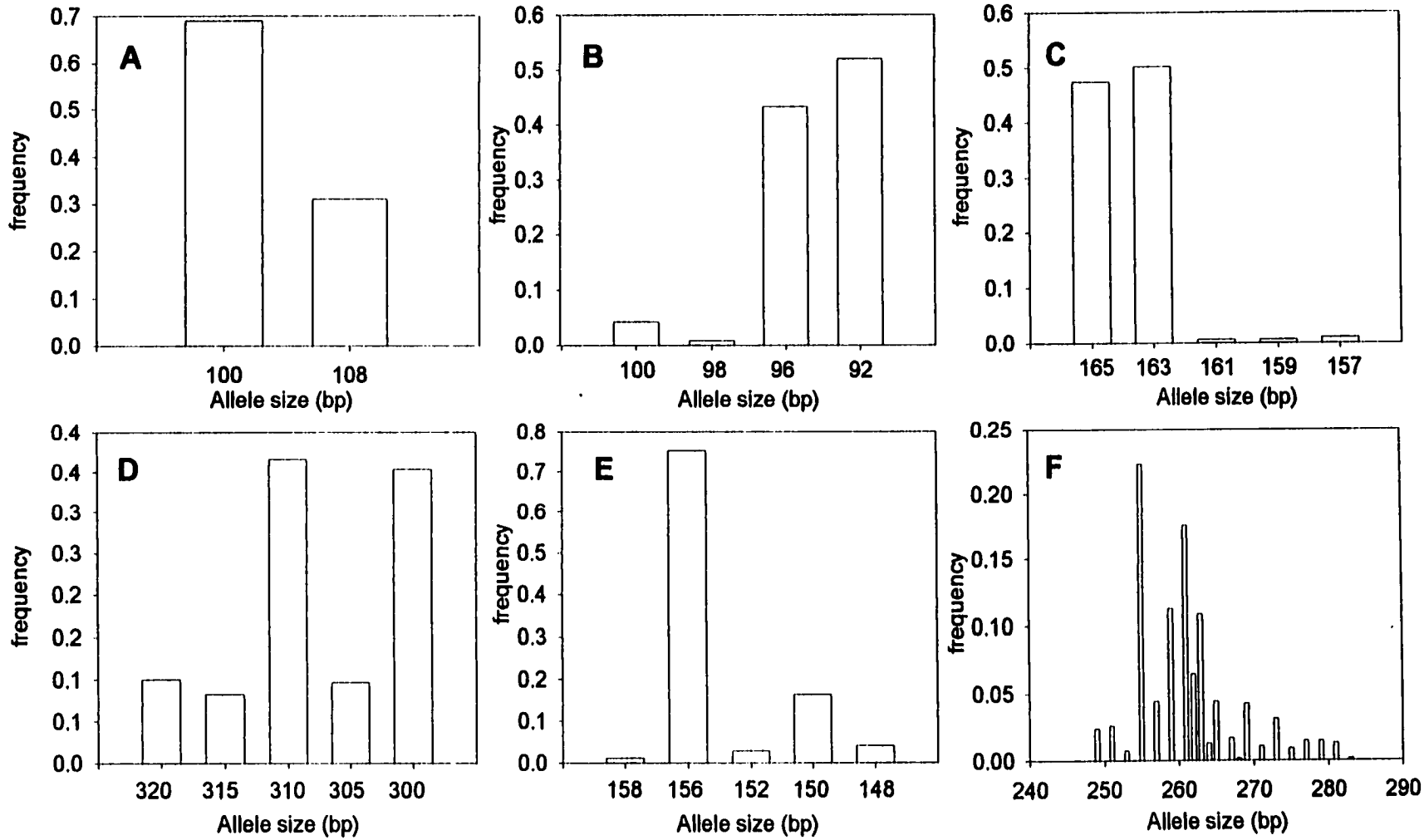


Figure 4.1. Frequency distributions of allele sizes observed at microsatellite loci used in the paternity analysis. Allele frequencies at *Pvc* 19 (A), *Pvc* 43 (B), H.g. 8.10 (C), β -g (D), PV 11 (E) and PV 3 (E) were calculated from adult harbour seals sampled at Sable Island, Nova Scotia between 1993 and 1995.

Genotype frequencies did not differ significantly between adult male and female samples across all 6 loci (genotypes with expected cell counts of less than 5 pooled, χ^2=10.078, df=22, P>0.05) nor did the genotype frequencies of adult seals differ significantly between the years of sampling (genotypes with expected cell counts of less than 5 pooled, χ^2=18.258, df=25, P>0.05), so adult male and female genotypes from all years were pooled to calculate population allele frequencies. All loci used in the analysis of paternity generally showed segregation patterns between females and pups consistent with a Mendelian pattern of inheritance of codominant alleles at autosomal loci, with a few noteworthy exceptions (see below). The distribution of observed genotypes was not significantly different from the distribution expected assuming HWE at each locus or overall (Table 4.3).

On 15 occasions a pup's genotype was inconsistent with the maternal genotype at a locus, involving 9 different female-pup pairs. For 3 pairs, the pups genotype was inconsistent with the maternal genotype at three loci (1994, n=1; 1995, n=2). It is unlikely that the 1995 mother or pup samples were switched or mislabelled since both female genotypes were inconsistent with either pup. The probability of detecting three microsatellite mutations occurring in the same meiotic event would be extremely low, hence the most parsimonious explanation is that the mother we sampled was not the biological mother. I therefore assumed that the pup was fostered, and I considered the maternal genotype to be unknown at all loci for paternity analysis. In the other 6 pairs, the pup's genotype was inconsistent with the maternal genotype at a single locus, either PV 3 (n=5) or β-g (n=1). In these instances, one of the pup's alleles differed by a single repeat unit from the most

similar allele in the maternal genotype, twice longer and 4 times shorter by a single repeat unit, which would be consistent with pre-zygotic slippage mutations of a single repeat unit during DNA replication in the maternal germ line. For three of the nine mismatches between pups and mothers which differed at more than 1 locus, the most similar alleles differed by more than 1 repeat unit. For these reasons, and since these loci were the most polymorphic of all loci I have tested, which suggests that they may have relatively high mutation rates, I considered that each inconsistency resulted from a maternal mutation and the female was the true biological mother. Assuming 5 mutations at PV 3 and 1 at β-g in a total of 260 observed meiotic events, the estimated rates of detectable mutations were 0.019 and 0.0038 per meiotic event for PV 3 and β-g, respectively.

Occasionally samples failed to amplify by PCR at a locus during the first amplification attempt (Table 4.4). This occurred more frequently when attempting to amplify the locus PV 3. If a sample failed to amplify on the first attempt, I repeated the amplification and usually the reaction succeeded. Following failure to amplify three times, a sample was re-extracted and PCR amplification repeated up to another 3 times. Samples from males and pups were extracted a third time if necessary since they are more important to the analysis of paternity, but logistical constraints meant that it was not possible to invest as much effort towards re-extracting samples from females. Eventually, logistical constraints limited the number of attempts I could make to amplify samples which repeatedly failed to amplify, and failures at PV 3 finally accounted for 32 of 61 total missing single locus genotypes. If, for paternity analysis, the maternal genotype was not known at a given

Table 4.4. Number of samples which repeatedly failed to amplify by PCR.

Type	Year	1 locus	2+ loci	% of all samples	% of all amplifications	Overall impact on paternity analysis
Males	1993	0	0			
	1994	1	0			
	1995	0	0			
	combined	1	0	1.1	0.2	-none, (sample was excluded)
Females	1994	13	6			-marginal, in 18 of 40 total cases none since
	1995	13	6			the pup was homozygous at the same locus
	combined	21	7	14.2	3.4	
Pups	1994	8	1			-increased proportion of ambiguous paternity
	1995	10	0			(average 47.1% vs 25.4% for pups typed at all 6 loci)
	combined	18	1	6.9	0.1	

locus, either allele present in the pup was considered equally likely to have been derived paternally. If the pup was homozygous, this had no impact on the likelihood calculation at that locus. If a pup's genotype was unknown, all males were considered equally likely to have provided the paternal allele at that particular locus(i.e. P ($A_p|G_m$) and $P_k(A_p)$ were set to 1). One male failed to amplify at PV 3 and was excluded from the paternity analysis.

Paternity analysis

The estimated probabilities of exclusion predicted the observed frequencies of paternal exclusions closely, and for each locus were not considerably different between the 1994 and 1995 cohorts (Table 4.5). The discriminating power varied greatly between loci with their information content, as PV 3 and β-g accounted for a greater proportion of the exclusions observed than the other less polymorphic loci. Overall, the probability of a randomly chosen male being excluded as a possible genetic father of a pup was estimated as 0.956 in 1994 and 0.947 in 1995. The average probability of selecting a pup's paternal genetic contribution from a randomly chosen paternal gamete using this genetic system was less than 0.003 in both years (Table 4.5).

I was able to identify a single most-likely sire for 64 pups among those sampled in 1994, and for 54 pups in 1995 (Table 4.6). In other instances, either all sampled males were genetically excluded at one or more loci (n = 48 and 39 in 1994 and 1995, respectively), or more than one male was the most-likely candidate (n=32 and 38 ambiguous cases in 1994 and 1995, respectively). The

Table 4.5. Genetic discriminating power of microsatellite loci in the analysis of paternity. Estimated probabilities of exclusion (P(ex)), frequencies of exclusions observed (F(ex)) and the average probabilities of obtaining the paternal genetic contribution from a randomly selected male ($P(g_x|G_r)$) of the 6 microsatellite loci used in the analysis of paternity in the 1994 and 1995 pup cohorts on Sable Island, Nova Scotia, are shown.

Locus	P(ex)		F(ex)		$P(g_x\|G_r)$	
	1994	1995	1994	1995	1994	1995
Pvc 19	0.158	0.181	0.143	0.165	0.543	0.531
H.g. 8.10	0.186	0.217	0.166	0.192	0.481	0.467
PV 11	0.310	0.204	0.282	0.171	0.496	0.555
β-g	0.485	0.476	0.469	0.470	0.271	0.268
Pvc 43	0.280	0.288	0.263	0.271	0.403	0.418
PV 3	0.777	0.720	0.771	0.735	0.107	0.158
Combined	0.956	0.947	0.957	0.937	0.0019	0.0027

Table 4.6. Results of paternity analysis for pups born in 1994 and 1995 on the north beach of Sable Island, Nova Scotia. Value in parentheses represent either percentage of all samples or the range of observed values.

	1994	1995	Test statistic	P
Pups sampled	144	131	na	na
Cases in which all sampled males excluded[1]	48 (33.3%)	39 (29.8%)		
Cases in which paternity ambiguous[1]	32 (22.2%)	38 (29.0%)	$\chi^2 = 1.68$[1]	0.43
Cases in which one most-likely sire found[1]	64 (44.4%)	54 (41.2%)		
Median number of sires per pup when all males not excluded	3 (1-25)	3 (1-25)	W = 3510[2]	0.26
Median LOD of most-likely candidate male	3.63 (2.47-7.28)	3.70 (1.77-6.73)	W = 3213[2]	1.00
Median ΔLOD between most and next most-likely candidate male	0.69 (0.69-7.28)	0.69 (0.69-6.73)	W = 3926[2]	0.48

1. Chi-square test for difference between cohort years in cases for which all males excluded, paternity ambiguous and one most-likely found.
2. Mann-Whitney test statistic for differences between cohort years.

two males which shared an identical genotype were not identified as the most-likely sire of any sampled pup. The median LOD of the most-likely sire was 3.63 (1994) and 3.70 (1995), which represent paternity odds of approximately 36 to 1. There were no significant differences between years in either the median number of most-likely sires I was able to identify, or the median LOD and ΔLOD between years (Table 4.6).

ΔLOD was bimodally distributed in both 1994 and 1995, which indicated that the genetic data either very strongly or only weakly implied paternity for the majority of the most-likely sires identified. The overall distribution of ΔLOD did not differ between years (χ^2 test, cells with less than 5 expected observations pooled, see Figure 4.2).

I estimated the mean total number of male harbour seals on the north beach to be 248 from the frequency of sightings of previously marked males during censuses I conducted on the north beach in 1993 and 1994 (Table 4.7). No attempt was made to judge the maturity of males during censuses, because harbours seals lack secondary sex characteristics which indicate sexual maturity (Boulva and McLaren 1979, Bigg 1969). I therefore assumed that approximately 20% of these males were reproductively immature. Male harbour seals become reproductively mature at 5 or 6 years of age (Boulva and McLaren 1979), and subadult males, which show little evidence of reproductive behaviour, are present in the colony during the breeding season (Walker and Bowen 1993ab). I therefore estimated the number of effective breeding males to be 200 for the purpose of estimating α probabilities using the PATSIM 1.1 simulation. There

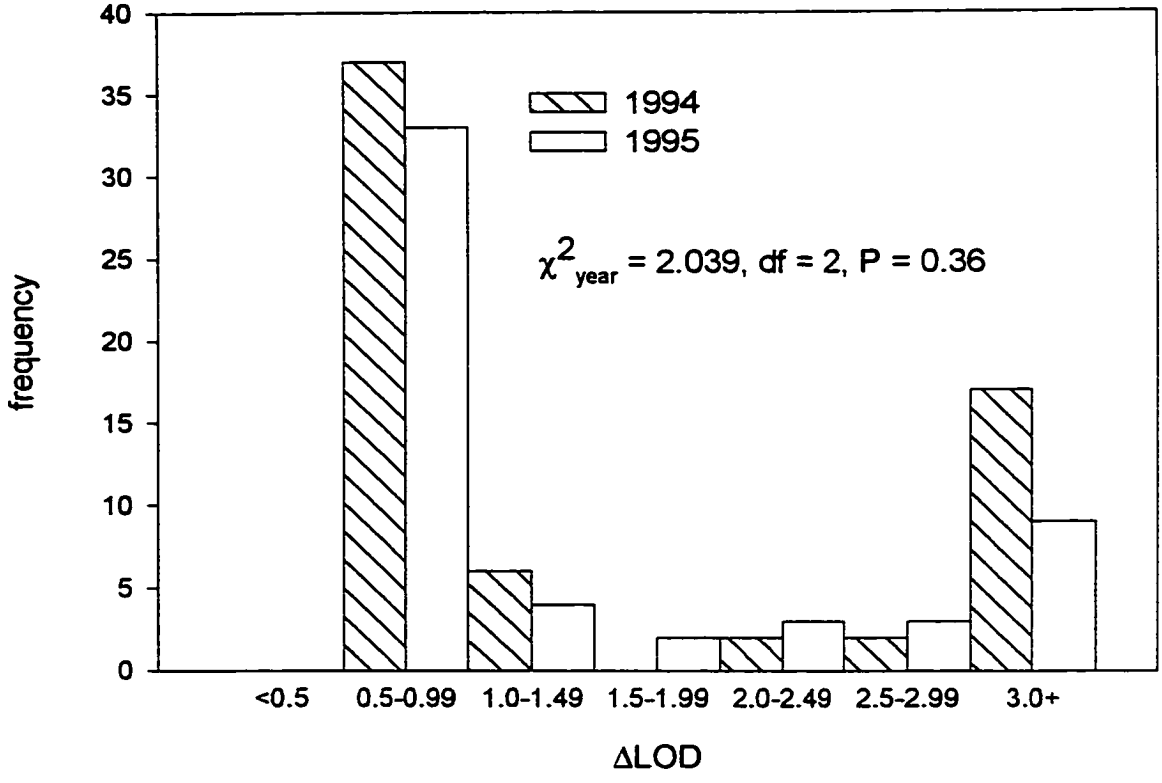


Figure 4.2. Observed frequency distribution of ΔLOD for male harbour seals. Data shown are for the 1994 and 1995 pup cohorts born on the north beach of Sable Island, Nova Scotia. Reduced degrees of freedom reflect combining small (n<5) cell sizes.

Table 4.7. Estimated number of male harbour seals on the north beach of Sable Island, Nova Scotia.

Census date	June 1, 1993	June 9, 1993	May 29, 1994	June 12, 1994	June 20, 1994	Mean
total males sighted	112	107	112	102	54	97.4
total marked males sighted	19	18	11	12	8	13.6
total number of marked males present in population	44	44	21	32	32	34.6
Estimated total number of males in population	260	262	214	272	216	248

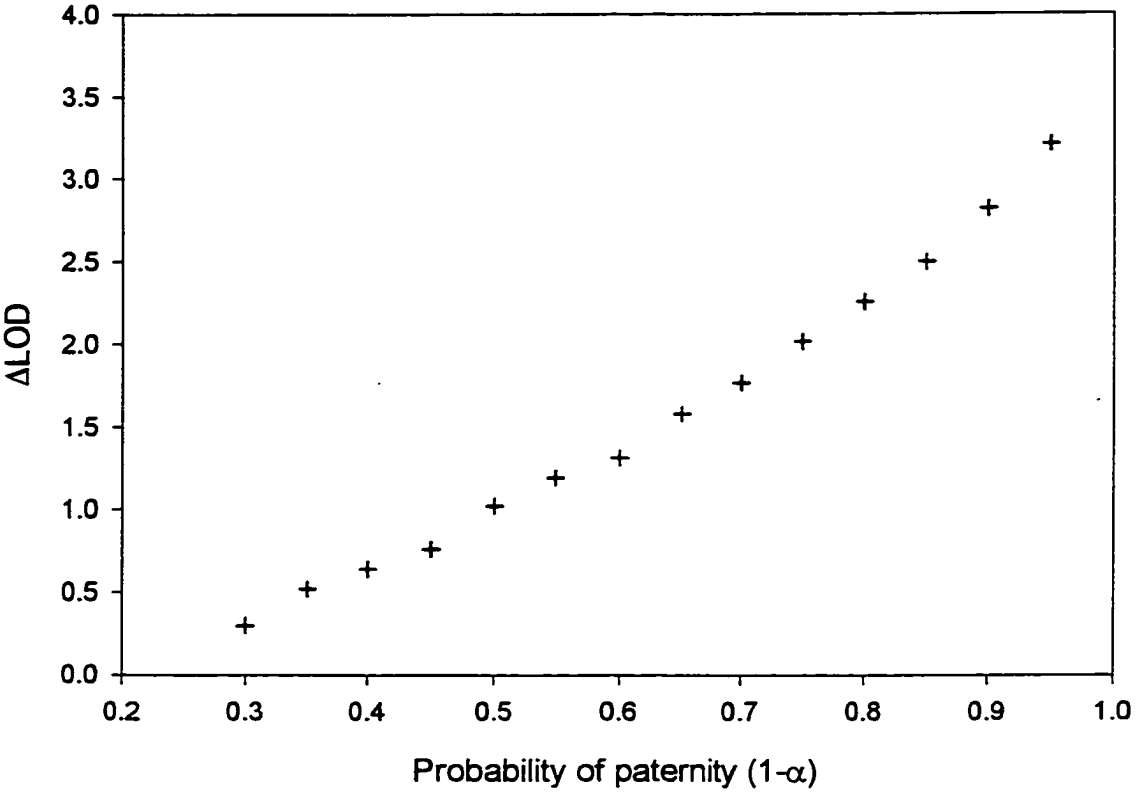


Figure 4.3. Critical ΔLOD generated by PATSIM 1.1 for a range of probability of paternity input parameters.

was a positive, slightly curvilinear relationship between the predicted critical ΔLOD generated by PATSIM 1.1 and the probabilities of paternity (1-α) (Figure 4.3). The frequency distributions of the probability of mating success (P_{MS}) were skewed to the right in both years, and the distribution of values did not differ significantly between the 1994 and 1995 cohorts (Figure 4.4A). The right skew reflects that most males were considered unlikely to have sired a pup in either cohort year. The median probability of mating success was 0.4 in both 1994 and 1995. The distributions of the maximum and most-likely number of pups sired by all sampled males in both years were also skewed to the right (Figures 4.4B and C). Most males sampled were unlikely to have sired a pup in a given cohort, and the maximum number of pups for which a single male was identified as the most-likely sire was 6 in the 1994 cohort (male id R356) and 5 in the 1995 cohort (male id R378).

In both years the variance in mating success among all sampled males was low (Table 4.8). Neither Var [MS_{ML}] nor Var [MS_{max}] differed significantly between years (Levene's test for homogeneity of variance, P = 0.30 and 0.26, respectively). Neither P_{MS}, MS_{ML}, nor MS_{max} were significantly correlated between 1994 and 1995 (Spearman rank correlation coefficients, P > 0.05 for all: r = 0.03 for P_{MS}, see Figure 4.5; r = 0.08 for MS_{ML} and r = 0.05 for MS_{max}, data not shown), indicating that any measure of male mating success in 1993 was a poor predictor of mating success in 1994.

In both 1993 and 1994, 45 of the 90 males sampled and included in the paternity analysis were actually sighted or handled on the north beach during

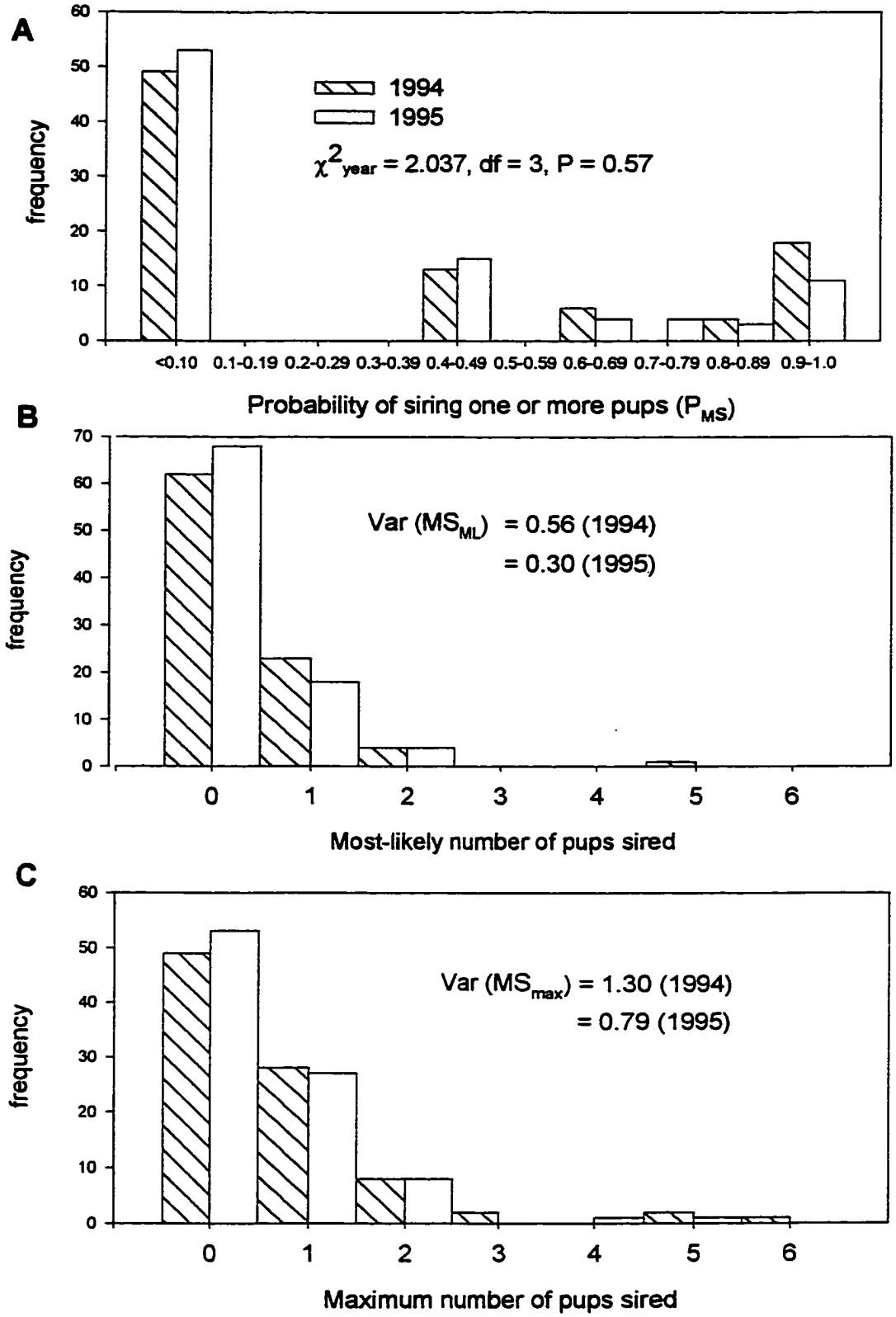


Figure 4.4. Frequency distributions of the probability of mating success (A), most likely number of pups sired (B) and maximum number of pups sired in the 1994 and 1995 cohorts born on the north beach of Sable Island.

Table 4.8. Variance in mating success among adult male harbour seals on Sable Island, Nova Scotia. Data are shown for 1994 and 1995 and for both years combined estimated by genetic paternity analysis.

	1994 Variance	1994 Variance/mean^2	1995 Variance	1995 Variance/mean^2	Years combined Variance	Years combined Variance/mean^2
Maximum number of pups sired	1.30	2.17	0.79	2.34	2.28	1.33
Most-likely number of pups sired	0.56	3.45	0.30	3.54	0.85	1.78

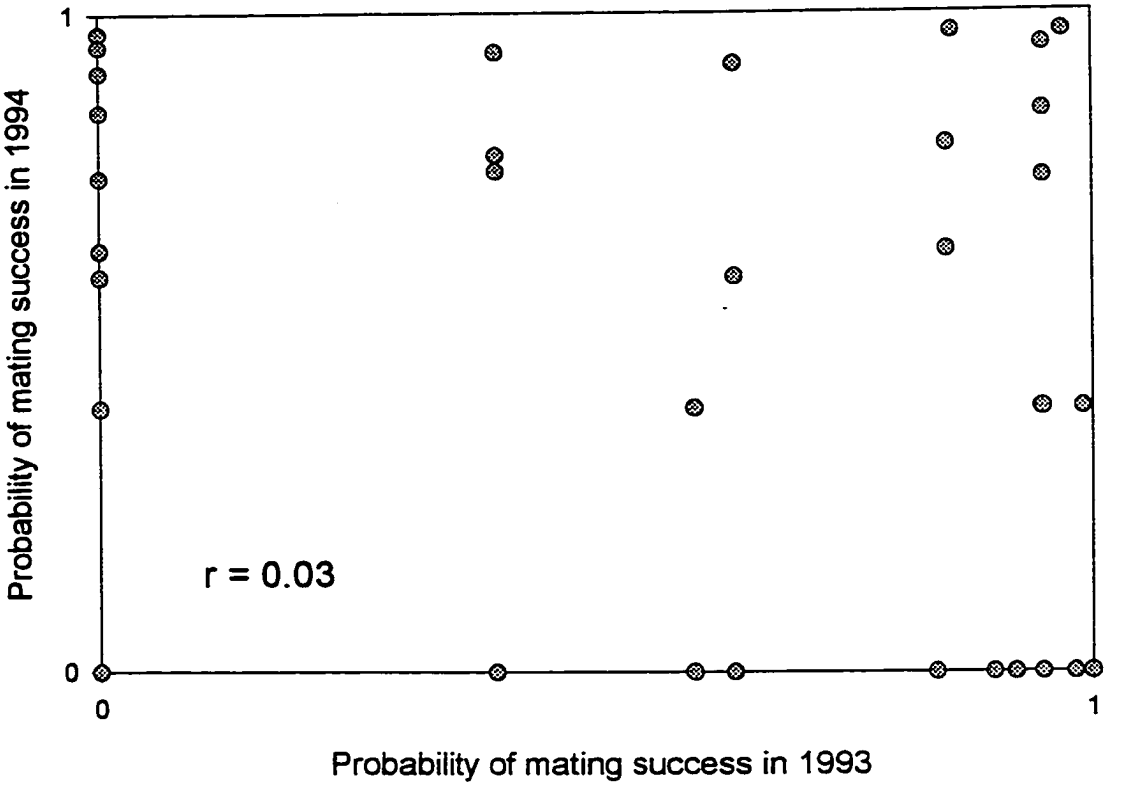


Figure 4.5. Probability of mating success in 1993 versus 1994 for all sampled males included in the paternity analysis (n = 90).

the breeding season. Neither the median P_{MS}, MS_{max}, nor the MS_{lik} differed significantly between males which were sighted and known to be present, and males which were sampled and assumed to be present, in either year (Kruskal Wallis ANOVA, df = 1 for each comparison, $P > 0.05$ for all).

Mating patterns

The genetic data suggested 118 total most-likely mate pairs for the 1994 and 1995 pup cohorts (Table 4.6). In 26 instances, both the female and the male were sighted on Sable Island in the previous year (n = 18 in 1993 and 8 in 1994), and their last sighted haul-out location noted during the course of other research. The distribution of the distances between the sighting locations of likely mates was not significantly different from a distribution of distances randomly generated by re-sampling all of the sightings of all males and females included in this study (Figure 4.6), suggesting that females did not necessarily mate with males who tended to use a nearby haul-out location. In four separate instances, a male with a very high probability of paternity for a given pup (≥ 0.95) probably mated with a female which was last sighted more than 10 km away from the male's last recorded haul-out location.

Pups born to the same female in successive years did not share significantly more paternally derived alleles than expected assuming females chose their mates at random (Figure 4.7), suggesting that the pups of females born in successive years are unlikely to be more closely related than half-sibs.

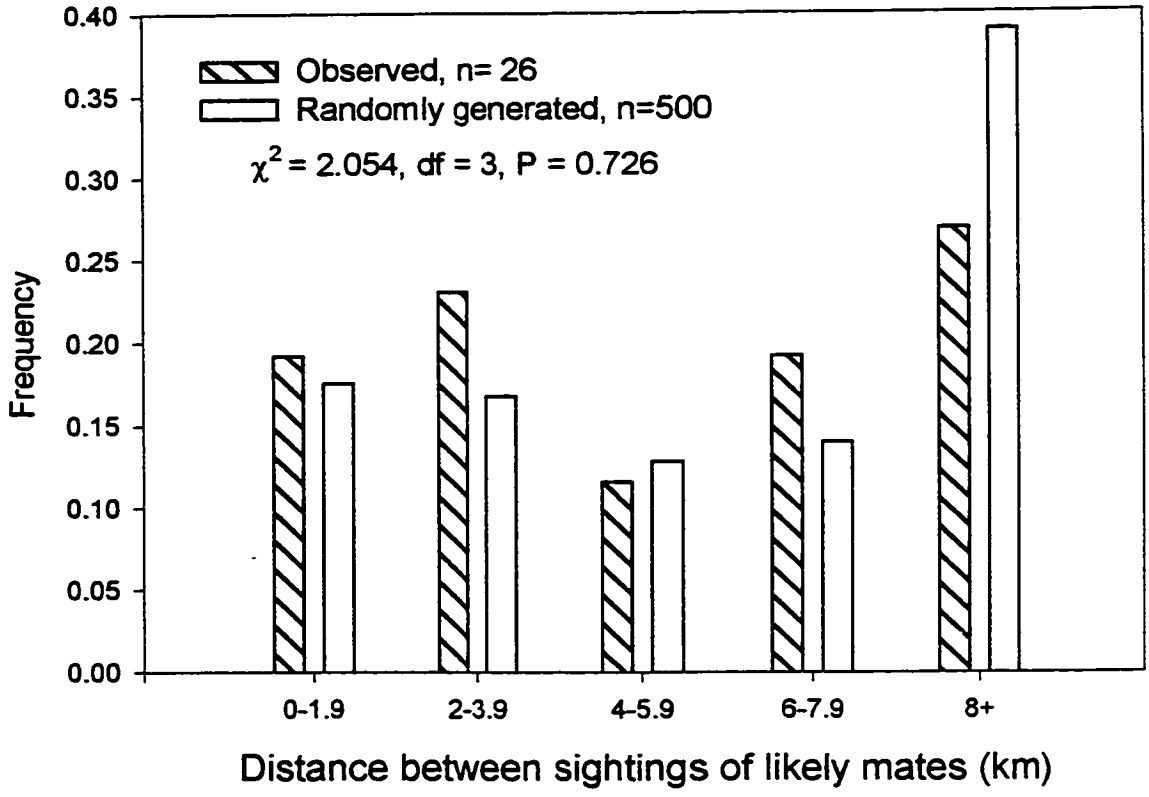


Figure 4.6. Distribution of observed distances between mate pairs. Data shown are the distances between the last sighted location of male and female harbour seals identified by paternity analysis, compared to a distribution of distances between male and female harbour seal locations simulated by randomly resampling male and females sighting data 500 times..

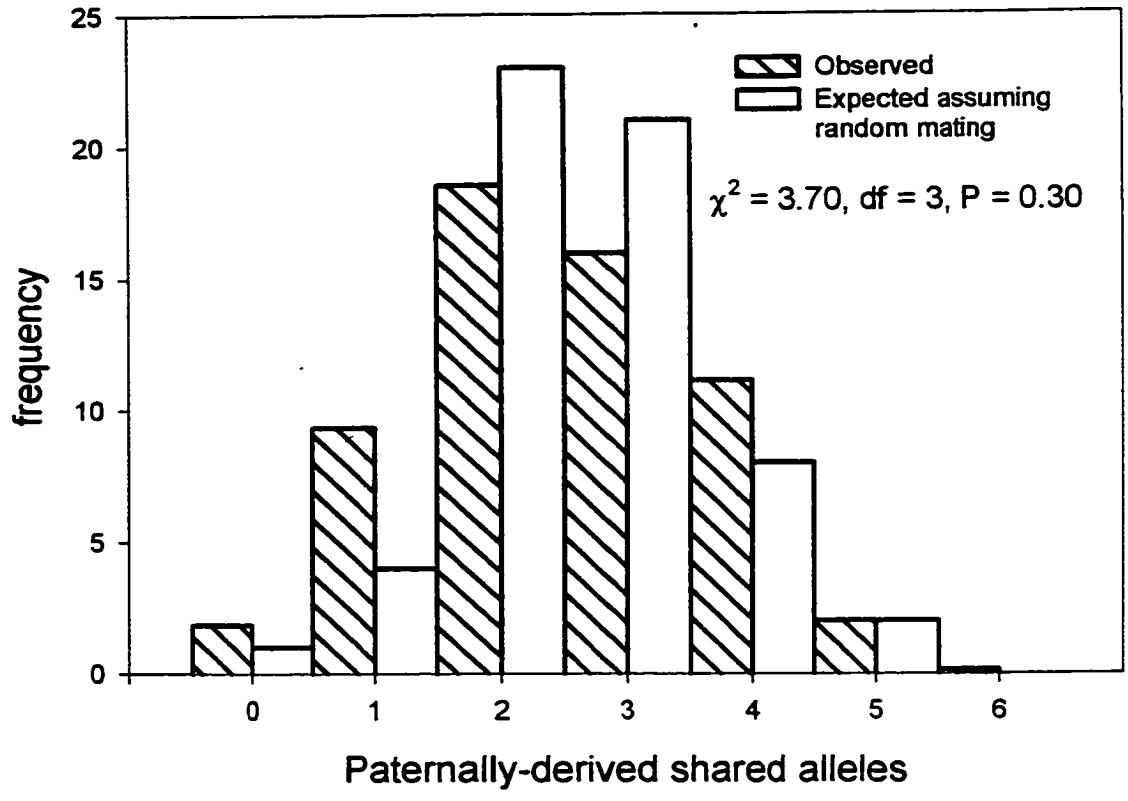


Figure 4.7. Relatedness between pups born to females in consecutive years. Distribution of the observed number of shared, paternally derived alleles in pups born to the same female in 194 and 1995 compared to the distribution of shared alleles expected assuming females choose their mates at random.

DISCUSSION

Performance of microsatellites in the analysis of paternity in harbour seals

Assuming that the sampled males included in the paternity analysis represent males which are equally successful as unsampled males, I would expect to find sires for a similar fraction (45%) of the sampled pups using an ideal genetic system. Paternity analysis identified most-likely sires for 44 and 41 % of the sampled pups in 1994 and 1995, respectively, however the degree of confidence that the identified male was the true sire, implied by the distribution of observed Δ LODs, was often relatively low (Figure 4.2). Furthermore, the average number of pups a male was expected to sire, assuming there were 200 breeding males, would have been 0.72 in the 1994 cohort sample (144 pups among 200 candidate males) and 0.66 in the 1995 (131 pups among 200 candidate males). These estimates are significantly higher than the median most-likely mating success for males in 1993 and 1994 (Wilcoxon one-sample test of the median; $n = 90$, $P<0.001$, for each year), suggesting that the genetic system may underestimate male mating success using the conservative measure of mating success (MS_{lik}). Similarly, all of the sampled males were genetically excluded approximately 30% of the time, and using an ideal genetic system this figure ought to be higher, perhaps close to 55% (the estimated proportion of unsampled males in the population).

The accuracy of the paternity analysis could be improved adding more polymorphic microsatellite loci to the paternity analysis, but there are currently

no other pinniped microsatellite loci available. Additionally, a complicating factor in this study was the relatively low level of genetic variability detected at microsatellite loci among harbour seals in the Sable Island population. Most loci I isolated appeared to have one or two alleles these harbour seals, yet they detected considerably higher levels of variability in all of the other pinnipeds tested (Table 4.2, Coltman **et al.** 1996). The primers developed by other laboratories also detected higher levels of genetic variability in other harbour seal populations in Europe and in European grey seals (S. Goodman, personal communication, and Allen **et al.** 1996) than in Sable Island harbour seals, as most loci appeared to have low heterozygosity and only 1 or 2 alleles in the initial screening. A discussion of the source of this lack of genetic variability is beyond the scope of this thesis, but it is an important factor contributing to the levels of confidence which were estimated for the identified most-likely sires.

Nonetheless, the combined discriminating power of the genetic system employed in this study, which is largely attributable to the highly polymorphic locus PV 3, is similar to that previously employed in published studies of paternity in other organisms. Morin **et al.** (1994) used 8 microsatellite loci with a combined P(ex) of 0.989 to determine paternity in a wild chimpanzee community, and the mean P(ex) of the system used in this study was 0.952. Also, Estoup **et al.** (1995) used 4 loci, generating a P(ID) of 3×10^{-5}, to estimate the number of males inseminating queen bumble bees, and the cumulative P (id) of the 6 loci used here was 6.6×10^{-5}. In any case, by using PATSIM 1.1 to formally estimate the degree of confidence associated with the paternal relationships suggested

by the genetic data, I directly incorporated the level of uncertainty in the measures of mating success which are used to describe the population variance in mating success, and in Chapter V to identify the phenotypic characteristics which are associated with the probability mating success.

For the purpose of broadly comparing the variance in male mating success between species, the estimates presented here may underestimate the true mating success of many males because of the resolving power of the genetic system, but this is a conservative and predictable error which can be accounted for by standardizing the variance by the mean (Wade and Arnold 1980) prior to making comparisons between species. Genetic estimates are also relatively unbiased compared to estimates of mating success based on behavioural observations. Direct observations can only be conducted during the day, therefore not all matings may be observed, and females may mate with multiple males and the assumption that the first copulation observed is the one which is successful may be incorrect (Boness **et al.** 1993).

Some authors have suggested that maximum likelihood methods of assigning offspring in paternity studies have an inherent bias towards individuals which are homozygous at many loci, because they will always have higher LODs than heterozygous individuals for a given offspring (Devlin **et al.** 1988). In this study, the number of homozygous loci did not have a significant effect on the median male P_{MS}, MS_{lik} nor MS_{max} in either cohort year (one-factor Kruskal Wallis non-parametric ANOVA; $n = 90$; $H < 3.89$; and $P > 0.20$ for all), indicating that male mating success was unbiased with respect to the number of homozygous

loci. The bias suggested by Devlin **et al.** (1988) may be mitigated by the fact that heterozygous individuals will, on average, be excluded as sires of fewer offspring. The probabilities of mating success in either year were not significantly correlated with the male genotype probabilities (Spearman rank correlation coefficient $r = -0.09$ and -0.16 in 1993 and 1994, $n = 90$, $P > 0.05$ for both), indicating that genetically determined mating success was also unbiased with respect to relatively rare or common genotypes.

Occasionally, samples failed to amplify by PCR, which is not unusual in itself, but because it occurred more frequently at one locus in particular (PV 3) it raises some questions about the cause. If samples failed to amplify due to null alleles; these are allelic variants which may a sequence mutation which prevents efficient binding of the primer (O'Reilly and Wright 1995), this would have a significant impact on the paternity analysis since many individuals which appear homozygous (one band) may be true heterozygotes (with a single null allele) (Pemberton **et al.** 1995).

Usually a sample amplified successfully on the first or second attempt following a failure, which suggests that the cause of the failure usually may have been due to chance events (such as relatively poor annealing of the primers to template in the first few cycles of PCR), minor inconsistencies in the reaction conditions, or contamination of the particular reaction mix. PV 3 may have failed more frequently for several reasons. First, it is a relatively large locus with many repeats, therefore it may amplify less efficiently by PCR than the other loci due to incomplete extension by *Taq* polymerase, or possibly from secondary

structure or folding in the regions flanking the repeats or within the repeat region itself. The flanking regions are also relatively rich in GC content, therefore the region of DNA containing the locus is relatively rich with hydrogen bonds and may denature less readily, or re-anneal prematurely in each PCR cycle. This could interfere with PCR amplification since *Taq* polymerase requires the template DNA to be single-stranded. In total, 15 samples from 288 adults repeatedly failed to amplify at PV 3 (frequency = 0.052). If each failure represented an individual homozygous for null alleles, the frequency of the null alleles can be estimated as $0.052^{0.5}$ = 0.23, assuming null alleles are in HWE. If null alleles were this common, it would cause a strong bias towards homozygotes in the observed genotype frequencies. The frequency of individuals which will appear homozygous but actually possess a single null allele would be 0.35 if the frequency of the null allele was 0.23. Since the frequency of homozygotes observed at PV 3 was only 0.18, which was only marginally, and not significantly, more than expected assuming HWE, it is unlikely that null alleles were common at this locus. Furthermore, in no instance did I observe a pup which appeared homozygous which possessed a genotype inconsistent with the maternal genotype. This would frequently occur if null alleles were transmitted between mothers and their offspring, and since the locus is highly polymorphic, it would usually be detected. It is more likely that failures to amplify at PV 3 may be a result of the reaction having greater sensitivity to minor variations in experimental conditions, such as the condition or concentration of template DNA in a particular sample.

The variance in mating success among male harbour seals

Since the levels of confidence in paternity were variable and sometimes low, I used several criteria to estimate the range in the estimated population variance in mating success. The variance in the maximum number of pups sired uses less conservative criteria (simply the most-likely sire) to estimate the upper limit of the variance among males. The variance in the most-likely number of pups sired accounts for the degree of confidence in paternity and attributed fewer pups to a smaller number of males. When standardized by dividing the variance by the mean number of pups sired per male squared, the variance describes the intensity of sexual selection on males in a population (Wade and Arnold 1980). This is a useful measure of the extent of polygyny when making comparisons between species or populations (see Clutton-Brock 1988 and chapters therein; Boness **et al.** 1993).

By either estimate, in both years the variance in mating success among sampled males was not high (Table 4.8), but some males may have sired up to 6 offspring in a single season. Thus harbour seals may be considered slightly polygynous. Variance estimates were slightly, but not significantly higher among males in 1993 than in 1994. The use of more conservative criteria for the variance estimate resulted in a higher estimate of the intensity of sexual selection on males in both years (Table 4.8). This follows because a larger proportion of males were considered unsuccessful, resulting in a reduced mean number of offspring sired per male.

Compared to data from other pinniped species, which exhibit a range of mating strategies both at sea and on land, the harbour seal falls at the lower end of a continuum of polygyny among the aquatically mating species (Table 4.9). By all measures, the mating success of males in the aquatically mating species are generally lower than in terrestrially mating species, but there is a wide range in the degree of polygyny evident among terrestrially mating pinnipeds (Table 4.9). The physical structure of the mating environment (i.e. aquatic versus terrestrial) is one variable which influences the distribution and defensibility of receptive females in time and space, but within each species and among populations there are many other ecological factors which are important determinants of the degree to which males can monopolize access to receptive females (Le Boeuf 1991; Boness 1991). In the case of Weddell seals and aquatically mating Juan Fernandez fur seals, there are relatively well-defined, defensible local resources (leads in the ice and sheltered thermoregulatory sites near the breeding colony, respectively) which attract potentially receptive females during the breeding season (Hill 1987 and Bartsh **et al.** 1992; Francis and Boness 1991). Males which successfully defend these sites either may have achieved more copulations than non-territorial males as inferred from coloured grease tranfer experiments in Weddell seals (Hill 1987; Bartsh **et al.** 1992) or attended as many females as did males which held terrestrial territories in the breeding colony (Juan Fernandez fur seals- Francis and Boness 1991).

Table 4.9. Comparison of male mating success among pinnipeds species with varying mating systems and environments.

	Harbour seal[1]	Weddell seal	Juan Fernandez fur seal[2]	Grey seal	Antarctic fur seal	Northern elephant seal	Northern fur seal
Mating environment	aquatic	aquatic	both	usually terrestrial	terrestrial	terrestrial	terrestrial
Predominant male strategy	unknown	resource defence	resource defence	female defence	resource defence	female defence	resource defence
Maximum seasonal mating success	6	8	8	6	19	90	161
$Mean_{MS}$[3]	1.19 - 1.47	2.5	2.3	3.0	9.2	17.5	56.3
Var_{MS}[4]	1.33 - 1.78	3.0	2	1.0	3.0	41.0	41.0
n	90	20	33	34	12	8	19
Method of MS estimate[5]	DNA	inference	observation	observation	inference	observation	observation
Original reference[6]	this study	Hill (1987)	Francis and Boness (1991)	Boness and James (1979)	Boyd (1989)	Le Boeuf (1974)	Bartholomew and Hoel (1953)

1. Parameter estimates for this study are shown as the range between the minimum values of MS_{lik} and upper limits suggested by MS_{max} averaged between 1994 and 1995.
2. Data shown for males which compete for mates aquatically by defending thermoregulatory sites off the beach.
3. Mean mating success among successful males (one or more matings).
4. Variance in MS standardized by divided by the $(mean_{MS})^2$ to facilitate comparisons between species.
5. DNA- molecular genetic fingerprinting, observation- mating inferred by direct observation of copulations; inference- coloured grease tranfer or by operational sex ratio.
6. Numeric data presented in this table were taken directly from Table 1 in Boness et al. (1993).

However, these examples may not be typical of other aquatically mating pinnipeds. Like harbour seals on Sable Island, female harp, hooded (Kovacs 1990; Boness **et al.** 1988) or crabeater seals (Siniff **et al.** 1979) are dispersed spatially during the breeding season. The variance in male mating success in these species may therefore be low as it is among male harbour seals on Sable Island.

Among the terrestrially mating pinnipeds, the degree of the spatial clustering of females within a specific colony has been identified as an important determinant of the extent to which polygyny occurs (Boness 1991). It strongly influences the number of females a single male can simultaneously attend, either by defending groups of females themselves or territorial boundaries. Female grey seals on Sable Island were widely dispersed compared to females in the other species shown in Table 4.9 due to the widespread availability of suitable pupping habitat (Boness and James 1979). This may explain in part the relatively low level of polygyny in grey seals compared to other terrestrially mating species.

Small clusters of female harbour seals were widely dispersed along the north beach of Sable Island during the 1993 and 1994 breeding seasons, and mating occurs at sea, thus it is not surprising that even the most successful males may have sired only a small number of pups. It would be very difficult for a male to predict female locations and movements if the predominate male strategy was either to intercept females near the beach in a home range, or to search for receptive females near the breeding colony.

The variance in mating success is indicative of the extent of polygyny within a population, but on its own, tells little about the mating strategies used by males in a population. Male harbour seals were no more likely to mate with females who were located nearby than those which hauled-out 5 or more km away (Figure 4.6), suggesting either a male search strategy (which predicts low var_{MS}) or a display strategy in which females are attracted to either dispersed or clustered (lek) mating territories or home ranges. It is less consistent with an aquatic territory defense strategy such as has been observed for male harbour seals at Miquelon (Perry 1993). At Miquelon, females must pass through narrow channels to reach the sea, and males appear to defend the boundaries of aquatic territories in these channels during the breeding season (Perry 1993). At Sable Island, there are no such physical obstacles which limit the access of females to the water or channel female movements.

A low variance in mating success is not consistent with a classical lek mating system, and leks are relatively rare among mammals (Clutton-Brock 1989; Davies 1991), but a low variance in mating success does not necessarily mean that females do not choose mates. Similarly, although there was no evidence that females mated with the same male in successive seasons, this does not suggest that female mate choice is unimportant. Possibly, a male's attractiveness to females may vary between years, for example, with his physical condition, or relative to other males that are present in a given year. Alternately, intrinsic male quality may not vary much among male harbour seals making female mate choice of low selective value.

The estimated mating success of males in one year was not a good predictor of mating success in the following year. Inter-annual variation in mating success may therefore have a homogenizing influence on the degree of polygyny implied by estimates of the variance in mating success when based on a small number of years. When I used the sum of pups sired in both years as a measure of overall mating success (Table 4.8), the estimate of the raw variance among males exceeded the estimate from either study year, however when standardized by the $(mean)^2$, the intensity of sexual selection is about one-half of that estimated in either previous year using either relaxed or stringent mating success criteria. Considerably fewer males were considered unsuccessful when both years of reproduction were taken into account, and the maximum number of offspring sired by a single male increased only by one, which suggests that the lifetime reproductive output among individuals males may vary less than single measurements of seasonal output would lead us to believe. Unfortunately, such data is very difficult to collect from organisms with reproductive life spans which exceed the lifetime of most research projects, and for pinnipeds lifetime reproductive success data is limited to a single species. Le Boeuf and Reiter (1989) estimated the intensity of sexual selection on Northern elephant seal males as 21.8 (n=91), while the single season estimate calculated from Le Boeuf (1974) was 41.0. The vast majority of the estimates of polygyny and the descriptions of mating systems of pinnipeds and other species are based on observations of a single season (references in Boness **et al.** 1993) and it is becoming increasingly clear that lifetime data on reproductive success is

necessary to gain a better understanding of selection and adaptation (Clutton-Brock 1988; Murray 1992). Seasonal data are important to the study of mating systems as they describe the processes which act within a single episode of selection (i.e. each breeding season), and seasonal data are also useful for identifying traits which may ultimately be subject to sexual selection pressure.

In summary, the genetic data suggests a low variance in mating success among male harbour seals relative to other pinnipeds, which is consistent with the predictions based on aquatic mating and the spatial distribution of females at Sable Island during the breeding season. The form of the mating system remains unclear, however. Males do not appear to mate more frequently with females which haul-out nearby, nor do females tend to choose the same mate in consecutive seasons (mate fidelity). The paternity data alone do not eliminate any mating strategies, but they more strongly suggest that males may search widely for mates, or that males may attempt to attract receptive females to more dispersed mating territories or home ranges by their aquatic displays. It may be possible to discriminate between these possible patterns by looking more closely at the phenotype and mating success of individual male harbour seals (Chapter V).

CHAPTER V

PHENOTYPE, MATING PATTERNS AND MATING SUCCESS OF INDIVIDUAL MALE HARBOUR SEALS

INTRODUCTION

Estimates of the variance in male mating success are useful for making broad inferences about the levels of polygyny within and among populations and species. However, to advance our understanding of mating systems and reproductive strategies, it is desirable to measure both fitness and phenotype of individuals, and to interpret these data in the context of ecological factors that influence the population in question (Boness et al. 1993). Many studies have examined the behaviour of individual male harbour seals during the breeding season such as mass change and feeding behaviour (Walker and Bowen 1993a), haulout behaviour (Sullivan 1981; 1982; Godsell 1988; Walker and Bowen 1993b) movement at sea from telemetry (Van Pariijs et al. 1996), underwater vocalizations (Hanggi and Schusterman 1994) and diving behaviour and energetics (Chapters II and III). However, male mating success was not known in any of these studies. One study attempted to directly relate variation in male behaviour to mating success in harbour seals. Using DNA fingerprinting, Perry (1993) found that three males which defended aquatic territories through which females had to pass to gain access to the sea, sired at least one pup.

However, the general conclusions of Perry's study are limited by the small sample size used in the paternity analysis.

Our current understanding of the reproductive ecology of harbour seals at Sable Island provides a framework for making predictions about the relative importance of male phenotypic traits to mating success. To recapitulate, the important phylogenetic and ecological characteristics are:

1) Males do not provide parental care.
2) Breeding is seasonal, therefore receptive females are clustered in time.
3) Females return to Sable Island to give birth and nurse their pups, therefore receptive females are spatially more clustered than at other times of the year.
4) Beach space is not limited, therefore breeding females are dispersed along the north beach of Sable Island.
5) Mating occurs in the aquatic environment, making it difficult for males to control female movements or to predict their locations at sea.
6) Females locations become less predictable as they start to make foraging trips of increasing duration in late lactation and following weaning their pups (Boness et al. 1994).
7) The access of females to foraging grounds does not appear to be limited by geographic obstacles which males may attempt to monopolize, such as the well-defined channels at Miquelon (Perry 1993).

From mating systems theory, I would predict either a male advertisement strategy, with males attempting to attract females to either dispersed or clustered (lek) mating territories or home ranges, or a form of female defence polygyny,

with males searching for and attending potentially receptive individual females sequentially (Clutton-Brock 1989). Walker and Bowen (1993b) suggested an intercept strategy for some males at Sable Island. They identified a group of males which hauled out independently of groups, and exhibited fidelity to particular geographic beach locationst. This suggested that these males focused their aquatic activity in nearby home ranges arrayed in parallel with the beach to intercept females as they depart and return from foraging trips. Data on the variance in male mating success and mating patterns presented in Chapter IV did not clearly support either clustered mating territories, since the variance in male mating success was low, nor the female intercept strategy since females did not appear to mate preferentially with males located nearby. More likely patterns may involve active searching for receptive females near the colony, or attracting females to a home range by advertising.

Regardless of the strategy employed by males to secure mates, breeding is an energetically expensive activity for male harbour seals (Walker and Bowen 1993a; Chapter III) which suggests that size and body composition (the amount of stored energy males possess), may strongly influence mating success. Harbour seals are slightly size dimorphic (McLaren 1993) suggesting that male body size is a characteristic which may be under weak sexual selection. Data presented in Chapters II and III suggests that larger male harbour seals, or males which are in better condition at the beginning of the breeding season, may have a competitive advantage because they can afford to spend more time making shallow dives associated with reproductive displays (Boness, Bowen,

Marshall and Buhleier unpublished data).These males may have higher encounter rates with potentially receptive females (Chapter II) and can afford to expend more total energy towards reproduction during the breeding season than smaller males (Chapter III). Therefore, in addition to identifying the general suite of phenotypic characteristics which are associated with reproductive success, I also sought to examine the influence of general body size and energetic characteristics on mating success. I hypothesized that seals which are the most successful start the season in the best condition, that is they have more stored energy initially, and this allows them to devote greater effort towards finding mates at sea. Other males need to spend more time foraging in the early part of the breeding season so they can energetically afford to compete for mates later when receptive females become more abundant.

In addition to examining diving behaviour and energetics, I also examined differences in serum testosterone profiles between groups of males with different behaviour patterns and mating success. In seasonally breeding species, peak serum testosterone concentrations usually occur prior to mating and induce the changes in physiology and behaviour which are associated with male reproduction (Crews and Moore 1986; Lincoln 1981). Data from captive male monk seals (Atkinson and Gilmartin 1992), hooded seals (Noonan et al. 1991) and from wild male Weddell seals (Bartsch et al. 1992) show similar patterns of testosterone secretion in male pinnipeds, where peak levels occurred prior to the time of mating then gradually diminished during the breeding season. Bartsch et al. (1992) found that dominant male Weddell seals maintained significantly

higher levels of testosterone through the breeding season than defeated or non-competitive males. In some species, it is thought that high serum testosterone levels may actually be maintained in response to winning social "challenges" or other aggressive behaviours (Harding 1981; Wingfield et al. 1990), but not in others (Moore 1987; Creel et al. 1993). Other studies have demonstrated positive correlations between serum testosterone levels and social dominance in field studies of mammals such as house mice, *Mus musculus* (Zielinski and Vandenbergh 1993) and sugar gliders, *Petaurus breviceps* (Stoddart et al. 1994). It is clear that elevated levels of testosterone can potentiate reproductive behaviour, but testosterone secretion is influenced by behaviour in response to stimulation of the hypothalamus, which implies that the circulating levels may also be a consequence of behaviour, particularly aggression (Harding 1981; Wingfield et al. 1990). I hypothesized that reproductively successful male harbour seals are socially dominant, therefore will show higher levels of serum testosterone than other seals, either as a cause or a consequence of their overall behaviour.

I sought to identify combinations of phenotypic characteristics associated with mating success by employing multivariate statistics to group males according to their probability of mating success (Chapter IV) and the measured components of male phenotype described in this chapter. The importance of initial body mass, energetics (Chapter III), aquatic behaviour patterns (Chapter II) and serum testosterone (this chapter) to mating success were examined by statistically comparing these characters between the identified groups of seals

with similar phenotypic and mating success characteristics. I also examined the influence on male mating success of male haul-out location, and of the extent to which males continue to return to the same location or move between groups within the colony.

METHODS

Study site and phenotypic data collection

The study was conducted during the breeding seasons (mid-May to the end of June) of 1993 and 1994 on the north beach of Sable Island (43°55'N; 60°00'W), a partially vegetated sandbar 160 km east of Halifax, Nova Scotia. Between 300 and 400 harbour seal females gave birth on both the north and south beaches of Sable Island during each year of the study.

In each year, adult (>85.0 kg) (Walker and Bowen 1993ab) male harbour seals were captured in nets as described in Bowen et al. (1992) near the beginning of the breeding season (from 20 May to 1 June). Seals were individually marked with fluorescent paint on the lower back to facilitate rapid identification during daily beach surveys and censuses, and two numbered rototags (Dalton, Henley on Thames) were applied to the hindflippers for permanent identification. Skin samples were taken from all males for the paternity analysis (Chapter IV). All seals were recaptured at least once near the end of the breeding season (20 June- 5 July). During all captures, males were weighed with a 200 ± 0.5 kg Salter spring-balance.

Surveys of the north beach were conducted daily at approximately 1700 to locate marked males. I recorded the haulout location relative to numbered stakes placed at 0.5 km intervals along the north beach, and the social context of the haulout group in which a male was sighted (1= alone, 2=mostly male group, 3=group containing mostly females and pups). At each capture and

during daily beach surveys the presence of bloody wounds on the neck and hindflippers of each male was also recorded (0=absent, 1=present). Approximately every 7 days, a census of all animals present in the study area was conducted and the size, location and composition (males, females, pups and juveniles) of all groups was also recorded. In addition, during cohort tagging the dates of birth of all pups born on the north beach in 1993 and 1994 was recorded, and this information was used to predict the temporal distribution of potentially receptive females.

In each year, a subset of the marked males were also the subject of more detailed studies of diving behaviour (Chapter II) and energetics (Chapter III). In addition to these males, which were recaptured at approximately 7-10 day intervals to investigate changes in body mass and composition with respect to the availability of oestrus females, I also attempted to recapture 12 additional males in 1994 at 7-10 day intervals to increase the sample sizes of the longitudinal mass change and serum testosterone profile data. A blood sample (10 cm^3) was taken from the extradural vein during each recapture of all males which were handled in the diving and energetics studies (Chapters II and II) and from these 12 repeatedly recaptured males for the testosterone assay. Males studied in 1993 and in 1994 were pooled for all statistical analyses since diving (Chapter II) energetic (Chapter III) and average mating success (Chapter IV) characteristics did not differ significantly between years.

Phenotypic variables and data analysis

For all marked males in 1993 and 1994, I calculated the following parameters describing various aspects of male phenotype for use in the multivariate analysis:

Social behaviour:

1) Proportion of times sighted alone.
2) Proportion of times sighted in mostly male groups.
3) Proportion of times sighted in groups with females.
4) Presence of bloody wounds indicating recent fighting.

Movement:

5) Index of haulout site fidelity, calculated as the standard deviation of the distribution of haulout site locations.
6) Proportion of days sighted.

Body size and energetics

7) Initial body mass.
8) Rate of mass change during the breeding season (final minus initial mass, divided by the interval between measurements in days)

The probability of siring one or more of the sampled pups born in the study area in the following year (P_{MS}, Chapter IV) was used as the measure of mating success. Other data were available for a smaller number of males, including dorsal length, diving behaviour (Chapter II), energetics (Chapter III) and testosterone (this chapter) and were not used in cluster analysis.

Cluster analysis was used with all 8 phenotypic variables and P_{MS} to identify groupings of animals with similar mating success and phenotypic characteristics. Prior to cluster analysis, all data were standardized to a mean of zero and standard deviation of one to ensure that all variables were measured on a similar scale. Initially, a hierarchical agglomerative cluster analysis of the Euclidean distances was performed using Ward's linkage to provide an initial data partition. Ward's linkage technique groups similar observations into clusters with to minimize the within-cluster sum of squares, until all observations are in a single unique cluster (Everitt 1974). A number of simulation studies have emphasized the accuracy of Ward's method over other hierarchical clustering algorithms for identifying true clusters in data sets (Jain and Dubes 1988), but there is no general consensus on which linkage method produces the most accurate results (Everitt 1974; Milligan 1980; Jain and Dubes 1988).

Since the exact composition of clusters identified by hierarchical clustering algorithms may be sensitive to outliers or intermediate observations in the original data (Milligan 1980), I used the major groupings identified by Ward's method as the starting point, or the initial data partition, for a second cluster analysis with the iterative K-means clustering algorithm. Once an observation is

placed in a group in hierarchical cluster analysis it cannot be separated later as more observations are added, so observations do not necessarily end up in the cluster containing the nearest centroid in Euclidean space. K-means recalculates the location of cluster centroids each time an observation is added or removed from a group, and places observations in the cluster with the closest centroid and repeats this procedure until no more observations can be moved and the pre-defined number of clusters consist solely of the closest observations (Minitab reference manual 1994). Monte Carlo simulations have demonstrated that the K-means method is most efficient at recovering true groupings in data when the starting data partition is close to the true solution (Milligan 1980).

Once groups of individuals were defined using cluster analysis on the entire dataset consisting of all marked individual males, I tested the hypotheses put forward in Chapters II and III by comparing the diving and energetic characteristics of the subset of males which were subjects in those studies between groups with varying mating success by parametric tests where possible (repeated measures ANOVA or ANCOVA models, and t-tests, where appropriate). All data were checked for normality and transformed if necessary prior to parametric statistical analyses, but if it was not possible to transform data to an approximately normal distribution, non-parametric Kruskal-Wallis ANOVA was used. Binary data, or tests of differences between observed and expected discrete distributions were tested using χ^2 goodness-of-fit tests. The significance of all statistical tests was set at $\alpha = 0.05$. All statistical analyses were performed with MINTAB Release 10 for Windows (Minitab Inc. 1994),

except for repeated measures models which were tested in SPSS for Windows version 6.1.3 (SPSS Inc. 1995).

Testosterone assay

Blood samples for the testosterone assay were taken as quickly as possible, usually within 5 minutes of capture, to avoid possible antagonistic effects of handling stress on circulating hormone levels. Blood samples were stored without preservative on ice in a cooler until they were processed at the end of the day. Serum was separated by centrifugation of whole blood for 30 minutes at 3500 rpm, and stored at -20°C until assayed.

Assays were performed within 4 months of collection in 1993 and 1994. Total serum testosterone was determined by a commercially available radioimmunoassay system (Testosterone/dihydrotestosterone ^{3}H Assay System, Amersham) following the manufacturers instructions. According to the manufacturer, the only steroid that has significant cross-reactivity to the antiserum is dihydrotestosterone (40-50%), hence the measurement of circulating androgen presented here represents total testosterone plus 40-50% of circulating dihydrotestosterone. In most other mammals, levels of circulating dihydrotestosterone are very low relative to testosterone (references cited in Testosterone/dihydrotestosterone ^{3}H Assay System manual, Amersham), but they have never been separated in a pinniped. I refer to the measured hormone levels simply as testosterone for simplicity.

Steroid hormones were separated by extracting serum diluted 1:4 in distilled water with diethyl ether. The mean extraction recovery of testosterone, estimated from adding serial dilutions of ^{3}H labelled testosterone standard to pooled harbour seal serum, was 92.7 ± 17.9% (n=12). Recoveries indicated that testosterone could be measured accurately in harbour seal serum using this system (T = 1.3 [^{3}H labelled added standard] - 0.12; $r^2 = 0.93$, n = 12, $P < 0.001$). The manufacturer reports the sensitivity of the assay to be approximately 3 pg/tube. Intra- and inter-assay coefficients of variation were estimated to be less than 10 and 17%, (n = 10 and 6, respectively). For statistical comparisons, serum T measurements were blocked into 4 equal time periods according to the date they were taken, and differences between groups of seals and over time were tested by repeated measures ANOVA. If a male had more than one testosterone measurement within a particular time period, the average serum testosterone concentration was used in the ANOVA model, and males lacking an observation in any time period were omitted from the statistical analysis.

RESULTS

Data describing the 8 phenotypic characteristics described above and P_{MS} (Chapter IV) were collected from a total of 68 male seals (39 in 1993, and 29 in 1994). Cluster analysis using Ward's linkage initially identified between 2 and 6 major groupings in the dataset (Figure 5.1) depending on where the branches separating groups in the dendogram are cut. I cut the dendogram along the axis indicated since this axis intersects the longest branches separating groups, leaving 4 major groups separated by relatively large distances. Using these groups as the initial data partition, K-means cluster analysis moved 7 observations from group B to group D to minimize the within-group distances to the centroid. The composition of groups A and C remained the same following K-means cluster analysis.

The phenotypic and mating success characteristics of the groups identified are shown in Table 5.1. Groups are characterized by a variety of different features. Group A seals resemble those identified as potentially successful males by Walker and Bowen (1993b), which tended to haul-out alone and show evidence of fighting. However, the members of this group are also have the lowest probability of mating success. Groups B and D exhibited similar patterns of social behaviour, they tended to haulout with females and show evidence of fighting, but they are distinguished from each other by initial mass, rate of mass change and the probability of mating success. Males in group B had the highest probability of mating success, were larger and lost more

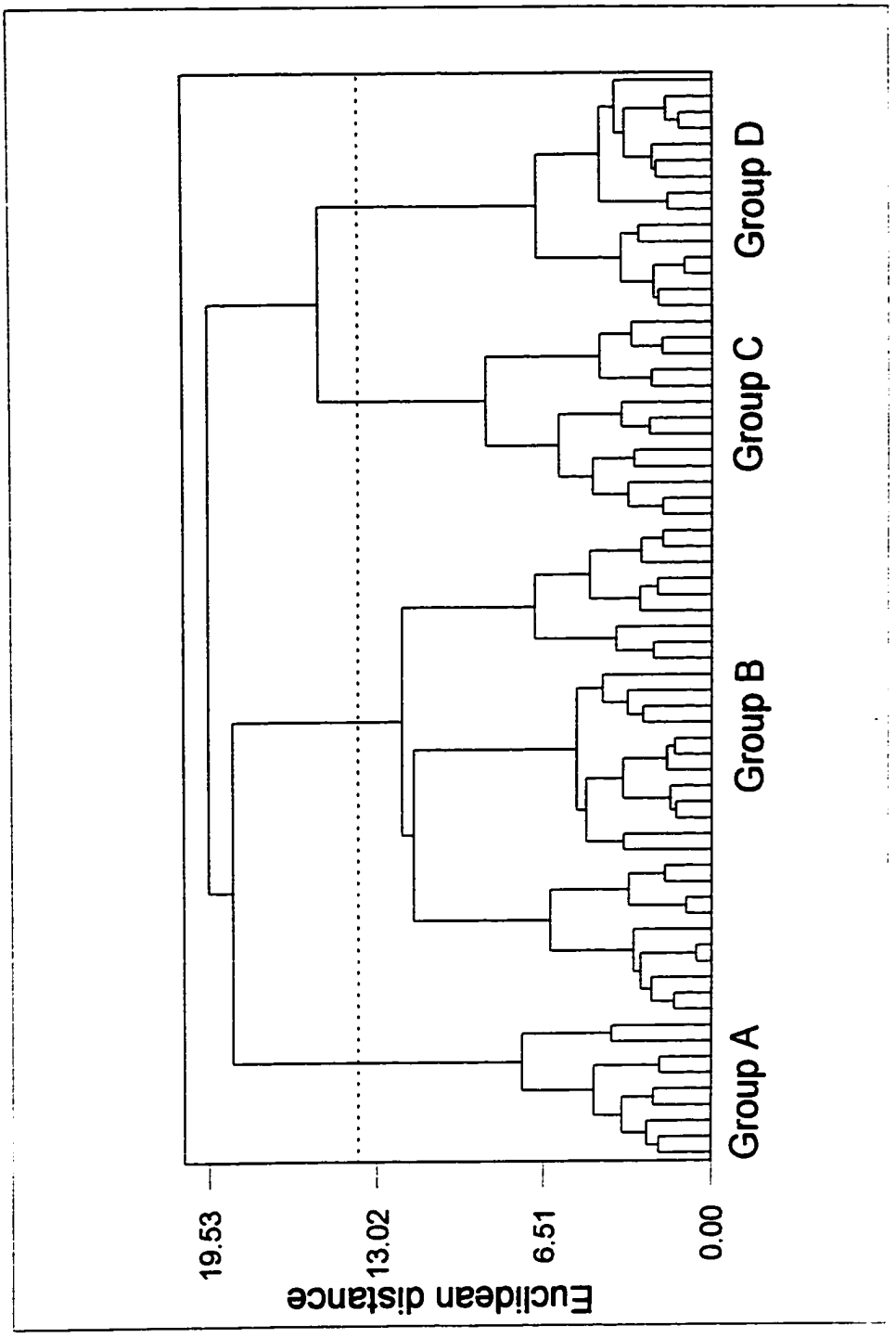


Figure 5.1. Denogram of observations produced by cluster analysis of male phenotype and mating success. Branch lengths reflect Euclidean distances between cluster centroids. The dotted horizontal line indicates the threshold used to define groups.

Table 5.1. Characteristics of groups of male harbour seals with similar phenotypic characteristics and probability of mating success identified by cluster analysis. Features which distinguish groups are shown in bold type.

	Group A	Group B	Group C	Group D	Test statistic (df = 3, 64)	P
Number of seals in group	9	24	13	22	na	na
Times sighted (% of days)	39.6 ± 4.9	35.0 ± 2.7	40.0 ± 3.0	45.6 ± 2.9	$F = 2.47^{\square}$	ns
Sighted alone (% of sightings)	**56.8 ± 5.1**	7.1 ± 2.2	6.5 ± 3.0	5.1 ± 1.4	$F = 31.1^{\square}$	<0.001
Sighted in male groups (% of sightings)	10.0 ± 4.9	26.5 ± 4.3	18.5 ± 4.8	8.9 ± 1.6	$F = 5.9^{\square}$	0.001
Sighted in mixed sex group (% of sightings)	**33.6 ± 5.3**	66.4 ± 3.8	74.9 ± 5.1	86.0 ± 2.1	$F = 21.1^{\square}$	<0.001
Haulout site fidelity index (km)	1.61 ± 0.62	1.53 ± 0.32	1.76 ± 0.49	1.75 + 0.27	F = 0.25	ns
Initial mass (kg)	107.6 ± 2.1	**110.5 ± 1.8**	103.6 ± 2.8	**99.6 ± 1.5**	F = 7.06	<0.001
Rate of mass change (kg/day)	-0.64 ± 0.06	**-0.71 ± 0.04**	-0.56 ± 0.06	**-0.44 ± 0.03**	$F = 6.2^{1}$	0.001
Evidence of fighting	7 / 9	24 / 24	**0 / 13**	22 / 22	$c^2 = 58.9$	<0.001
Probability of mating success	**0.09 ± 0.06**	**0.58 ± 0.08**	0.22 ± 0.10	0.25 ± 0.08	$H = 14.76^{2}$	0.002
Maximum number of pups sired	**0.22 ± 0.15**	**1.00 ± 0.16**	0.31 ± 0.13	0.59 ± 0.23	$H = 12.59^{2}$	0.006
Most-likely number of pups sired	**0.00 ± 0.00**	**0.63 ± 0.12**	0.23 ± 0.12	0.27 ± 0.12	$H = 12.32^{2}$	0.006

Notes: ns- not statisically significant; $^{\square}$- ANOVA following arcsine square-root transformation.

1. ANCOVA with initial mass as covariate.
2. Kruskal Wallis non-parametric ANOVA.

mass during the breeding season than males of other groups. Group C males behaved similarly to groups B and D, but did not show visible evidence of wounding from fighting with other males.

More detailed data describing dorsal length, changes in the rate of mass loss in relation to the availability of oestrus females, diving behaviour from TDRs (Chapter II) and energetics from isotope dilution experiments (Chapter III) were available for a variable number of males within the groups identified by cluster analysis (Table 5.2). I pooled mass change, testosterone, diving and energetic data from groups A, C and D for statistical comparison with the high mating success group (B) since the small number of observations in some groups made analysis by repeated measures ANOVA or ANCOVA inappropriate. However, mean data from each group are shown in all figures.

All males lost mass at a significantly higher rate in the mating period than they did prior to the appearance of oestrus females (Figure 5.2, repeated measures ANCOVA, average mass included as covariate, $F_{time} = 53.4$; df = 1, 28; $P < 0.001$). Group B males had the highest rate of mass loss in the pre-mating period (one-factor ANCOVA; $F_{group} = 4.6$; df = 1,30; $P = 0.04$), but the average difference between group B and other males was not quite statistically significant overall ($F_{group} = 3.09$; df = 1, 27; $P = 0.09$). However, data combining changes in body composition with mass loss indicate that males from group B expended significantly more of their initial energy stores per day during the breeding season than other males (Figure 5.3, repeated measures two factor ANCOVA, average mass included as a covariate, $F_{group} = 8.66$; df = 1,20; $P = 0.008$) and

Table 5.2. Sample sizes of detailed characteristics for statistical comparisons between groups.

Group	Total	Changes in rate of mass loss[1]	Four measures of serum T[2]	Diving behaviour	Changes in body composition	Changes in body water turnover
A	9	6	7	1	3	2
B	24	10	11	8	8	6
C	13	5	5	3	3	2
D	22	11	13	7	9	7
All	68	32	36	19	23	17

1. Observations for which there are independent measures of the rate of mass loss from 2 mass measurements in both the pre-mating and mating periods.
2. At least 2 separate measurements prior to 8 June, and 2 after 8 June.

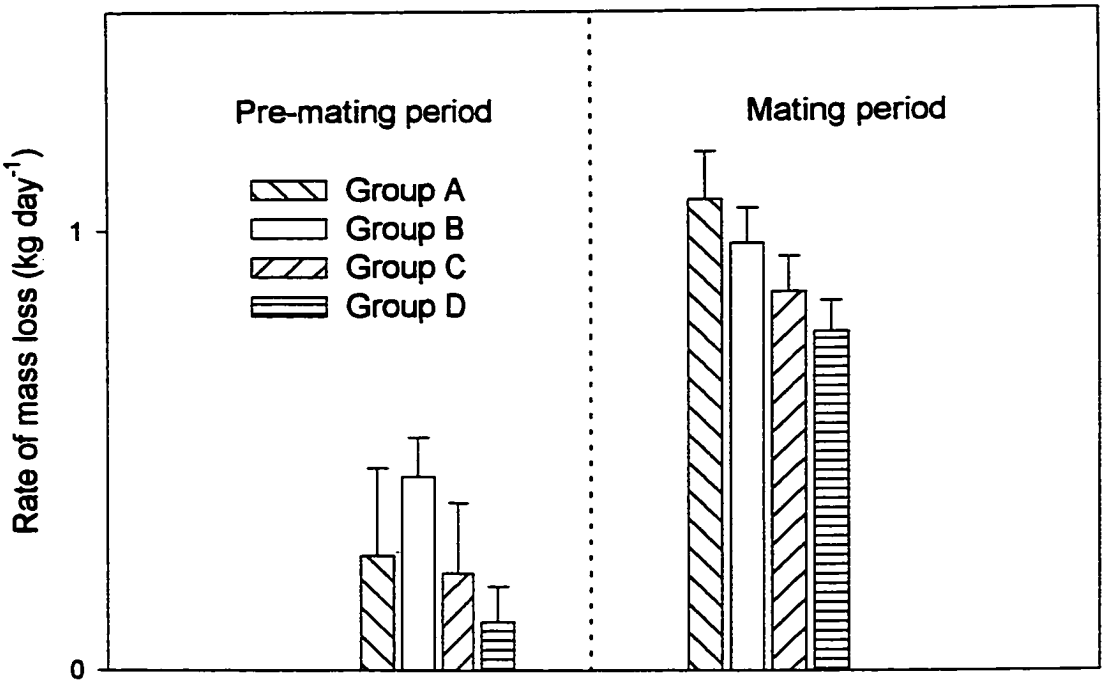


Figure 5.2. Rates of mass loss of male harbour seals in the pre-mating and mating periods of the breeding season. Error bars represent standard error.

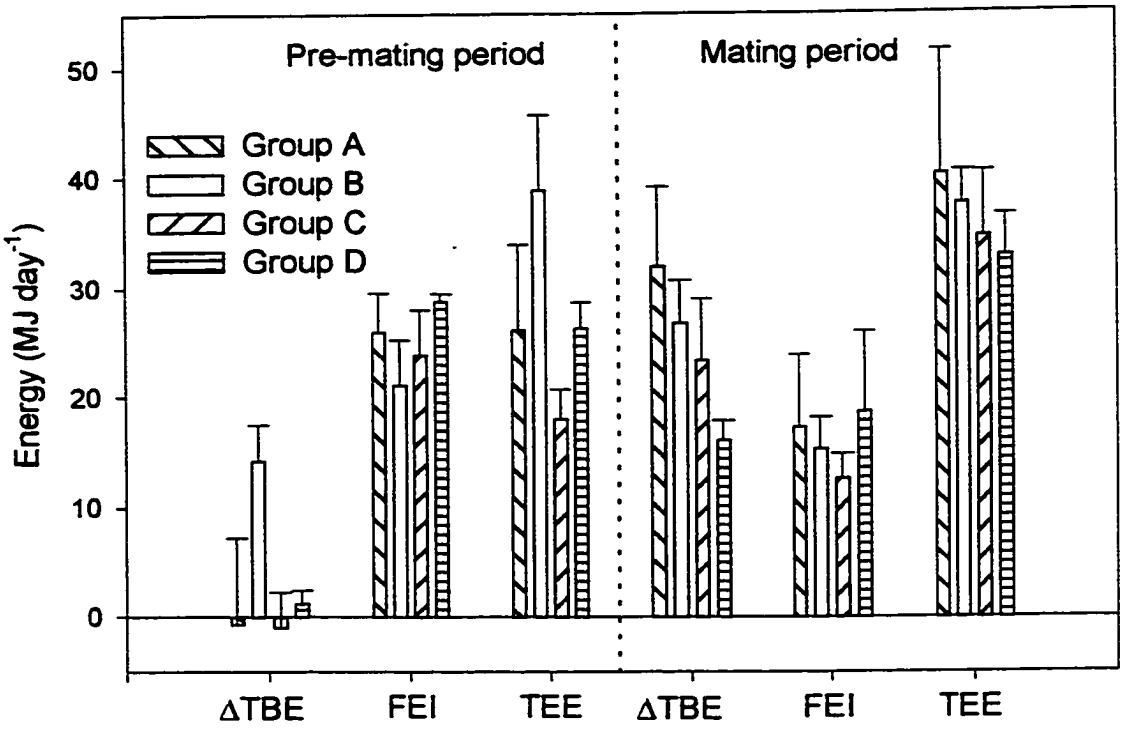


Figure 5.3. Energetic characteristics of males grouped by mating success and other characteristics. Rate of change in total body energy (ΔTBE) food energy intake (FEI) and total energy expended (TEE) of male harbour seals in the pre-mating and mating periods of the breeding season are shown.

this difference was most pronounced in the pre-mating period. All males lost significantly more total body energy per day in the mating period (F_{time} = 19.42; df = 1,21; P < 0.001). Estimated food energy intake did not vary significantly between group B males and other seals (two-factor repeated measures ANOVA, F_{group} = 0.32; df = 1,15) but was significantly lower overall during the mating period of the breeding season (F_{time} = 7.35; df = 1,15; P = 0.016) than the pre-mating period for all males. Combining both sources of energy, group B males expended significantly more total energy per day during the pre-mating period than other males (Figure 5.3, one-factor ANCOVA, average mass included as a covariate; F = 8.50; df = 1,15; P = 0.011) but not during the mating period (Figure 5.3, one-factor ANCOVA, average mass included as a covariate; F = 0.05; df = 1,15; P>0.05). Males in group B were composed of significantly more total energy at the beginning of the breeding season (1703 ± 99 MJ vs 1388 ± 55 MJ; t = 2.78, n = 8 and 15 for group B and other seals, respectively, P = 0.018). They contained approximately 22.7% more total body energy than other seals. Group A and B males were longer than males in Groups C and D (mean dorsal lengths were 154±2.1 [n=8], 155.7±1.3 [n=11], 151.8±2.1 [n=8] and 152.3±1.4 [n=14], respectively) but variation between groups was not statistically significant (one-factor ANOVA, df = 3, 37; F = 1.24; P = 0.31).

Group B males differed significantly from other males in shallow and deep diving characteristics with respect to the availability of oestrus females (Figures 5.4), however, there were no significant differences in the average time males spent hauled-out or at the surface between groups or between the pre-mating

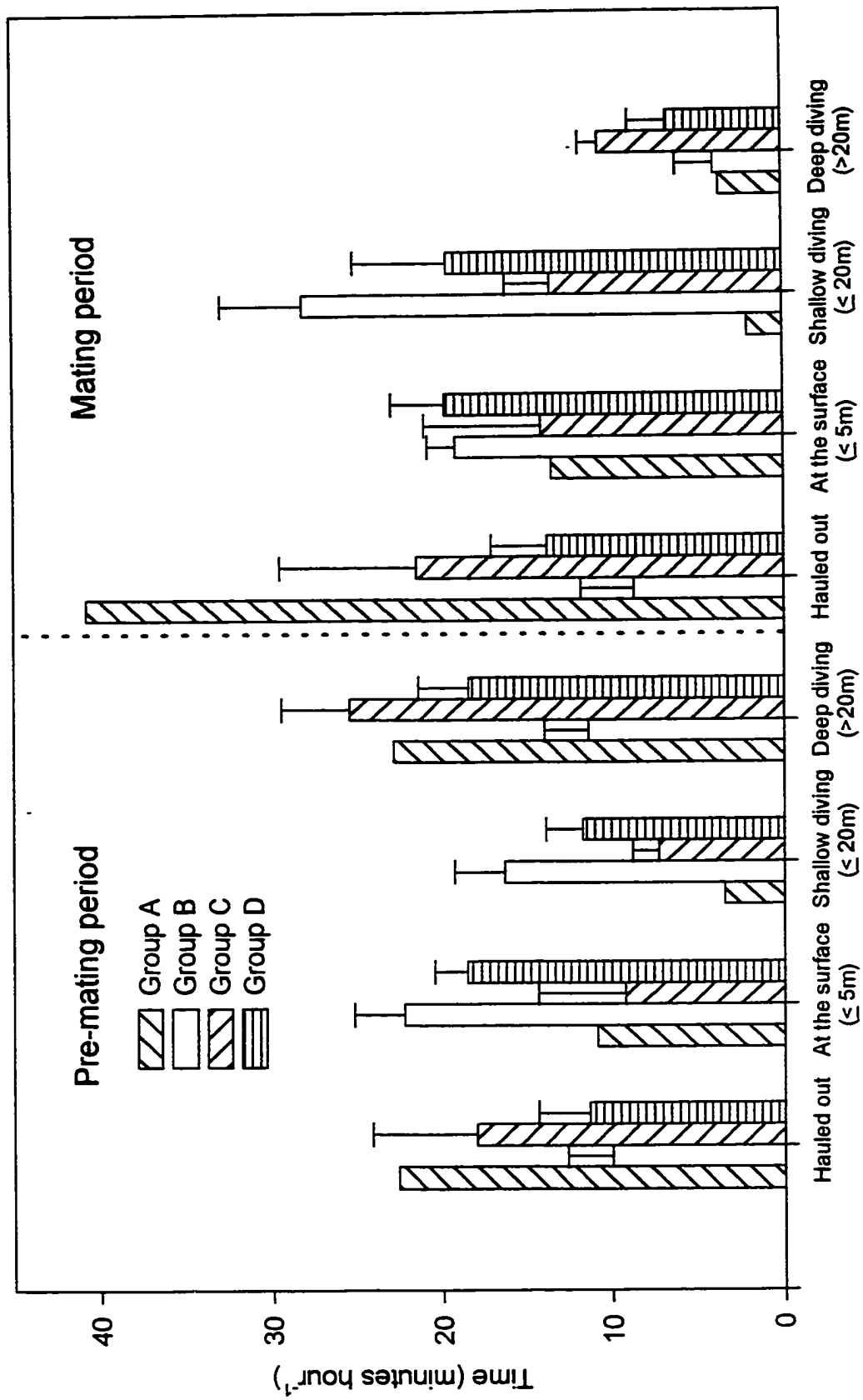


Figure 5.4. Activity patterns of male harbours seals at sea during the pre-mating and mating periods of the breeding season. Bars represent standard error, a single dive record was available for Group A males.

and the mating periods (one factor repeated measures ANOVA following arcsine-square root transformation; df = 1,17; F_{group} = 3.26 and 1.79; F_{time} = 0.07 and 0.00; for time spent hauled-out and at the surface, respectively; $P > 0.05$ for all). All males spent less time diving deeply, and more time shallow diving during the mating period than the pre-mating period (repeated measures ANOVA, F_{time} = 13.83 and 24.00; df = 1,15; $P < 0.002$ for both shallow and deep diving, respectively) but group B males spent significantly less time in deep dives and more time diving to 20 or less than other males during the entire breeding season (repeated measures ANOVA, F_{group} = 5.66 and 4.43; $P < 0.05$ for both deep and shallow diving activity, respectively).

Serum testosterone profiles

Serum testosterone concentrations in all groups declined significantly from peak concentrations of approximately 4.5 ng/ml at the beginning of the breeding season to levels of about 1.5 ng/ml near the end of the season (repeated measures ANOVA; F_{time} = 34.09; $P < 0.001$; Figure 5.5). There were no significant differences between the testosterone levels of Group B and other males (F_{group} = 0.45; df = 3, 81; P = 0.51), but the profile of group A seals appears to decline to minimal levels earlier (June11-21) than observed for other seals (June 22 - July 5, Figure 5.5).

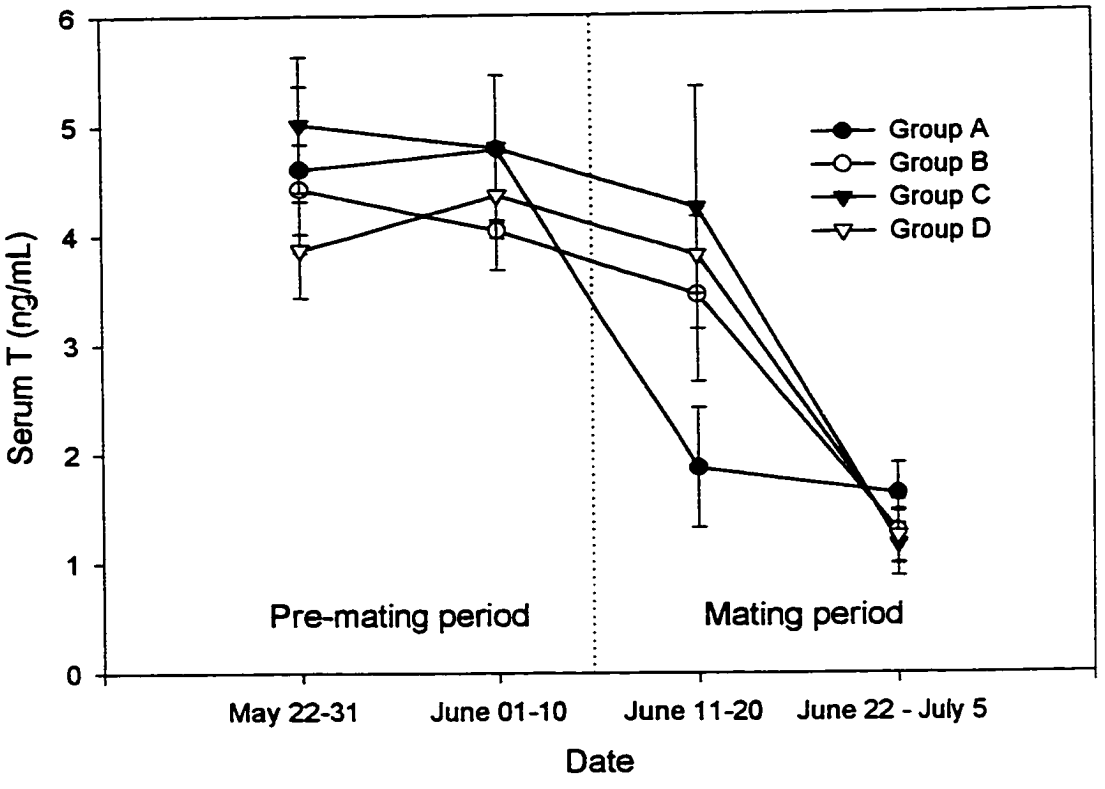


Figure 5.5. Serum testosterone profiles of adult male harbour seals during the breeding season on Sable Island, Nova Scotia. Means are shown ± standard error.

Male haul-out locations in relation to the spatial distribution of females

Male and female harbour seals were not uniformly distributed along the north beach when hauled-out, but tended to cluster at several predictable locations in both years (Figures 5.6 and 5.7). Three major concentrations of females were located at the 8.-10, 12-14 and 18-20 km regions of the beach in both seasons. Most males with high probabilities of mating success also frequently hauled-out near these locations (Figures 5.6 and 5.7), but they did not necessarily mate with females who were located in these nearby groups (Chapter IV). Group B, C and D males tended to haul-out near concentrations of females, whereas Group A males always tended to haul-out near the periphery of the colony in both years (Figures 5.6 and 5.7).

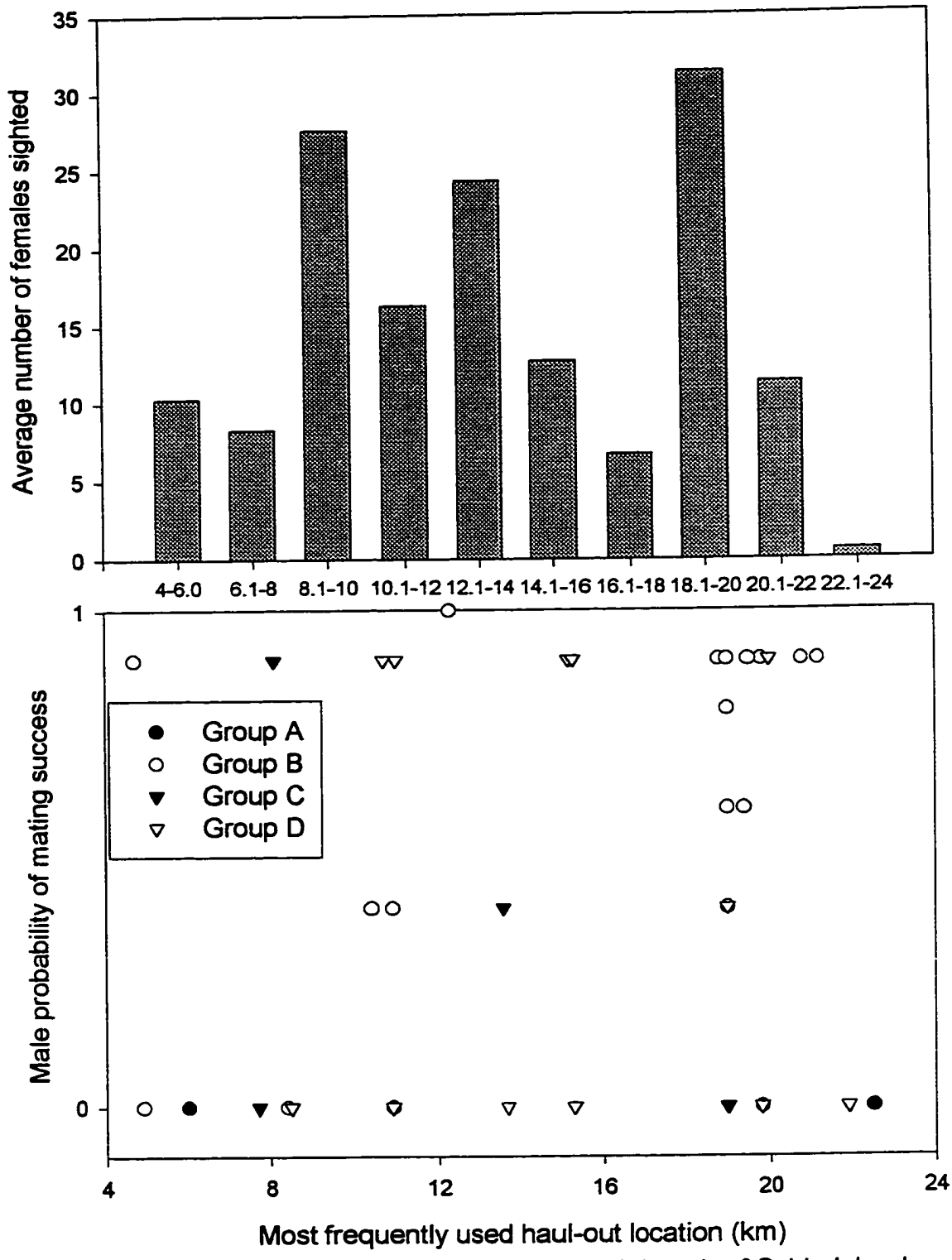


Figure 5.6. Distribution of females on the north beach of Sable Island. Data are from 3 beach censuses conducted in 1993 (top panel) in relation to the probability of mating success of males in Groups A, B, C and D plotted versus their most frequently used haul-out location (bottom panel).

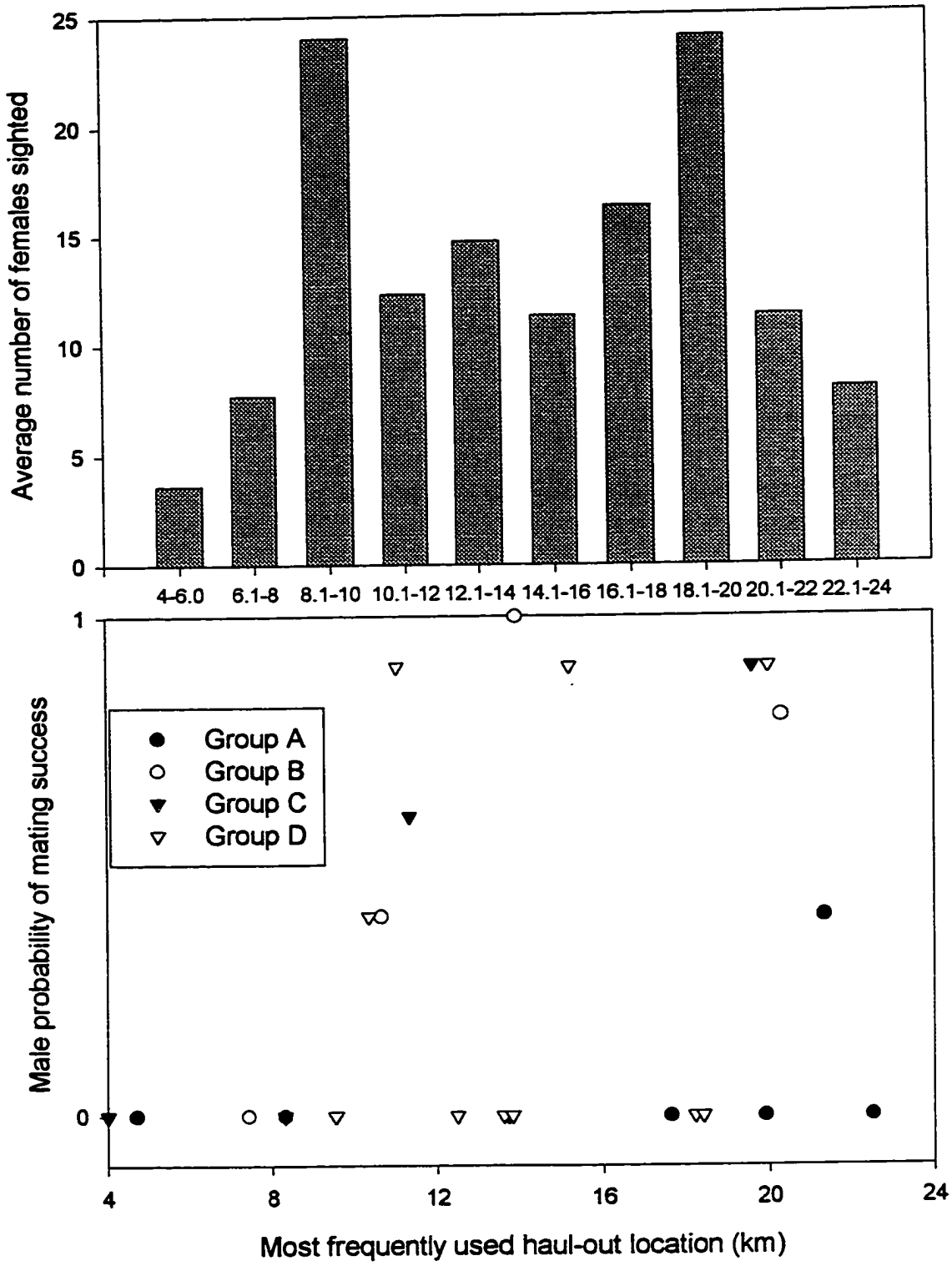


Figure 5.7. Distribution of females on the north beach of Sable Island. Data are from 3 beach censuses conducted in 1994 (top panel) in relation to the probability of mating success of males in Groups A, B, C and D plotted versus their most frequently used haul-out location (bottom panel).

DISCUSSION

Performance of cluster analysis and the exact composition of male groups

The final groupings produced by K-means cluster analysis may be sensitive to the initial data partition (Milligan 1980; Jain and Dubes 1988), therefore it is important that the initial partition be as close to the true solution as possible to have confidence that the groupings are biologically meaningful. I also used average, median and complete linkage techniques to generate initial data partitions. Average and median linkage techniques produced dendograms which were more difficult to interpret because they produced "straggly" clusters; they tended to chain observations together in one large group rather than distinct clusters. Complete linkage produced a similar initial data partition as did Ward's linkage, with several distinct clusters of males. Using an initial data partition of 4 groups generated by average, median or complete linkage hierarchical cluster analysis, K-means clustering always produced final groups of data which had very similar characteristics to the 4 groups shown in Table 5.1. In all cases, cluster analysis identified a small group of males with low mating success which usually hauled-out alone (i.e. group A), a group of smaller males with low rates of mass loss and little evidence of fighting (similar to group C), and two groups which differed in body size and mating success characteristics (groups B and D).

Patterns of phenotype and mating success

Group A males resemble the group of males described by Walker and Bowen (1993b) which were hypothesized to be reproductively successful. These animals frequently hauled-out independently of groups and showed evidence of fighting, but they were reproductively unsuccessful. One possible interpretation is that these males behaved differently from other males, in that they hauled-out near a small aquatic home range which they patrolled to intercept females as they move to and from nearby haul-out locations in the breeding colony. Their low mating success perhaps results from the fact that females are widely dispersed and there is no evidence that they tend to mate with males located nearby than other males (Chapter IV). A more likely explanation is that these males have been defeated in intrasexual competition, and they may have stopped competing for mates. The serum testosterone data, while not statistically significant, does suggest that their circulating testosterone levels had fallen off before males of other groups (Figure 5.5). This has been observed in male Weddell seals which appeared to have been defeated in intrasexual competition, presumably in response to elevated stress resulting from defeat Bartsch et al. 1992). These males may haul-out alone near the periphery of the colony in order to minimize further aggressive interactions. The single male in Group A for which diving data is available (R370) behaved in a very different manner from all other males. R370 spent significantly more time hauled out, on average 35.4 minutes per hour (greater than the mean of 13.9 minutes; $t = 9.76$;

$P < 0.001$) and considerably less time making shallow dives (2.34 minutes per hour vs. the mean of 16.41; $t = 6.10$; $P < 0.001$) over the entire study period. The differences in his activity were most pronounced during the mating period of the breeding season (Figure 5.4), when he spent almost 70% of his time out of the water. This strongly suggests that he was no longer competing for mates. In the early part of the breeding season this male associated with a group containing females, then abruptly started hauling out alone within 100m of the group's location on June 12th. At the same time, fresh wounds were sighted on his hindflippers which suggests that he had been fighting, and perhaps received the wounds while being chased by another male in a losing encounter.

Males in groups B, C and D had similar social behaviour characteristics; they rarely hauled-out alone and they were most frequently sighted in groups containing females. There were no significant differences between groups in the degree to which males exhibited haul-out site fidelity (Table 5.1). Groups C and D are distinguished from group B by their lower mean initial masses, rates of mass change, and probabilities of mating success. Groups C and D differed from each other primarily by the presence of wounds, which is difficult to interpret since it does not necessarily indicate that group C males do not fight or compete aggressively. There was no significant difference in initial body mass ($t = 1.25$; $df = 18$, $P = 0.23$) length ($t = 0.18$; $df = 13$; $P = 0.86$) or the mean rate of mass change between groups C and D when average mass was accounted for (ANCOVA; $F = 2.97$; $df = 1, 34$; $P = 0.10$). Nor where there marked differences in their diving behaviour (Figure 5.4), which suggests that they competed equally

intensely for mates. Therefore Groups C and D may not represent males which differ meaningfully in their phenotype.

Group B males represent males which are most likely to be reproductively successful in relation to other groups; their median probability of mating success was 0.69, whereas the median mating success groups C and D was 0 (Kruskal Wallis non-parametric ANOVA; $H = 10.63$; $n = 2$; $P = 0.005$). There are no pronounced differences is their haul-out behaviour, but they are heavier on average (Table 5.1), and they had more total energy at the beginning of the breeding season than other males. The difference in initial total body energy between groups can be attributed to variation among males in initial body mass (ANCOVA initial mass as a covariate; $F_{mass} = 45.93$; $df = 1, 18$; $P < 0.001$), therefore group B males are not composed of more energy relative to their mass at the beginning of the season. But, after variation in body size has been accounted for, group B males expend significantly more total energy during the breeding season, particularly in the pre-mating period (Figure 5.3), and this difference can be attributed to greater mobilization of total body energy rather than food intake. Group B males can therefore afford to spend more time making shallow dives associated with reproductive displays and aggressive interactions in both the pre-mating and mating periods, and they spend less time diving deeply than other males (Figure 5.4). The differences in energetic and diving behaviour characteristics between group B and other males are most strongly pronounced during the pre-mating period. Group B males start to energetically invest in reproductive effort earlier than other males (Figure 5.3). As they spend

more time making shallow dives and less time making deep dives which must occur away from the colony, males in group B may encounter more oestrus females in shallow water near the shore early and throughout the breeding season (Figure 5.4).

This may provide them with a mating advantage for several reasons. First, evidence from histology and oestradiol profiles suggest that parturient females are likely to become sexually receptive before the time of the appearance of the first weaned pups in the colony. From the timing of the appearance of luteinized follicles in the ovaries of lactating female harbour seals, Boulva and McLaren (1979) suggested that females may ovulate up to 2 weeks prior to the termination of lactation. Similarly, serum oestradiol concentrations monitored in captive harbour seals showed peaks indicating oestrus to occur about one week prior to weaning (Reijnders 1990). Assuming that parturient females may become sexually receptive 7 days prior weaning their pups, based on the distribution of observed births at Sable in 1993 and 1994,a mean lactation duration of 24 days (Muelbert and Bowen 1994), and a period of receptivity lasting 4 days, I estimate that up to 30 % of the parturient females in the Sable Island population could become sexually receptive during the pre-mating period (Figure 5.8).

Secondly, previously non-pregnant captive female harbour seals have been found to exhibit peak oestradiol concentrations approximately 2 weeks earlier than lactating females (Reijnders 1993). Therefore, if previously non-pregnant females visit Sable Island to mate, many of them may become

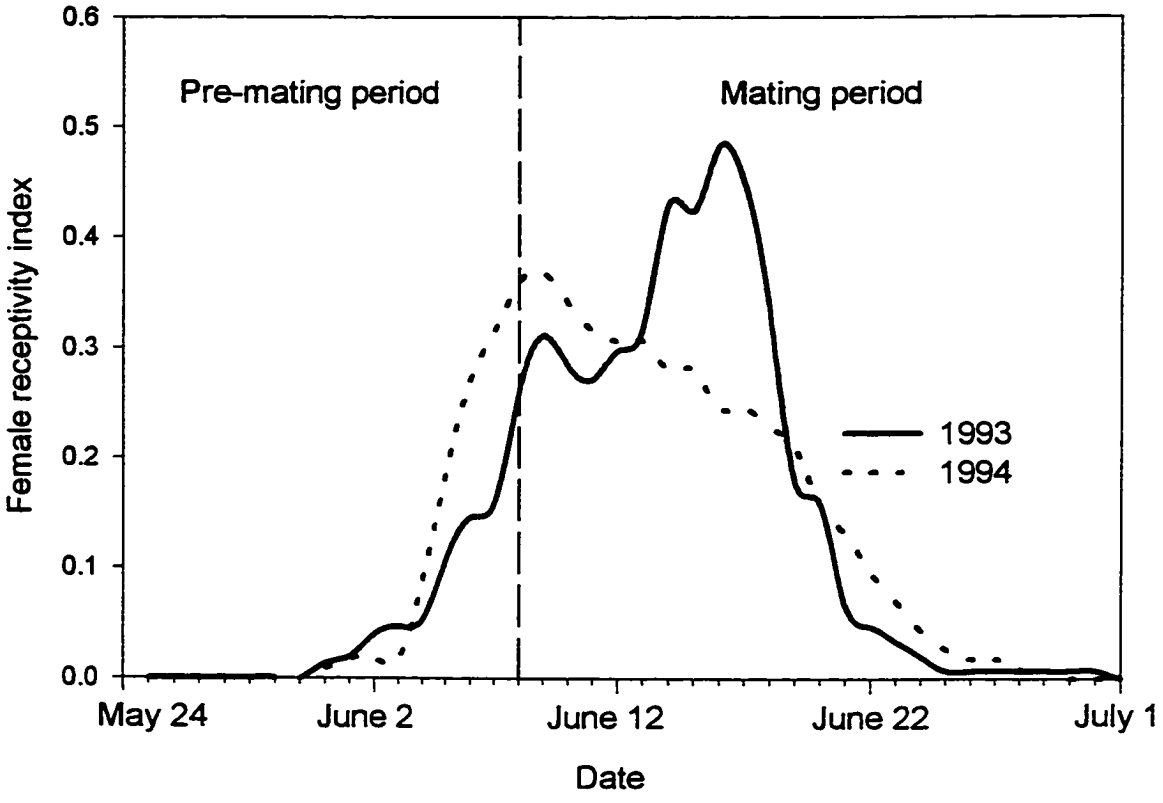


Figure 5.8. Distribution of receptive females in time during the pre-mating and mating periods of the breeding seasons of 1993 and 1994. The period sexual receptivity was taken as days 17 to 20 postpartum.

receptive during the pre-mating period of the breeding season. Group B males, which appear to have started advertising or searching for receptive females earlier than other males, will have a mating advantage because they will be more likely to encounter these females when they are receptive.

Group B males were on average, larger and more successful than other males in other groups, however there was considerable variation in both phenotype and mating success within groups. Not all males in Group B were relatively large at the beginning of the breeding season, in fact, 3 of 10 males in Group B with P_{MS} greater than 0.95 were less than 100 kg at the beginning of the breeding season. One of these individuals (R202) was a water-flux study animal, and was fitted with a time-depth recorder. These experiments suggested that he competed intensely for mates in the pre-mating period of the breeding season. R202 spent little time either shallow or deep diving, but more time at the surface than average in both the pre-mating and mating periods. Despite having less than average initial stored energy (1394 MJ), during the pre-mating period R202 expended approximately 39 MJ of total energy per day, and 50% of this energy came from body stores. R202 also was seen bearing fresh wounds from fighting on the neck relatively early in the season (June 1, 1993).

There were 11 relatively large males (>108 kg), 3 of which were classified in group A, which usually showed evidence of competing early, but were considered unsuccessful ($P_{MS} = 0$), hence initial body mass and condition cannot be considered a reliable predictor of mating success. As well, some reproductively successful males, classified in group C, were relatively small,

showed no evidence of wounding during the season, and made many deep dives while maintained their mass during the pre-mating period (R362 and R237).

Individual variation between years

Six males were studied in both years, and in 4 cases they were classified in the same group in both years (twice in group B, once in group D and once in group A). In the other 2 cases, the individual was classified in group B in 1993 and in group D in 1994. In all cases, there were no notable differences in their behaviour between years. The male classified in group A hauled out alone frequently while the others continued to associate with groups, and all 6 used a haul-out location within 1 km of that used in the previous year. Males did not differ significantly in their initial mass (paired t-test; $t = 0.25$; $df = 5$, $P > 0.05$) nor rate of mass change ($t = 0.12$; $P > 0.05$) between years. The two males which were classifed in group D in 1994 were not identified as the most-likely paternal candidates of any pups born in 1995; whereas they were both possible sires of pups born in 1994 although the genetic evidence was not strong ($P_{MS} = 0.40$ for both). The males classified in groups A and D and one of the males classified in group B in both years were considered unsuccessful (P_{MS}) in both years, and the other group B male had a very high probability of mating success in both years ($P_{MS} = 0.97$). The genetic data suggested that he sired 2 pups from 2 different females in each year. These observations suggest limited variation in overall behaviour between years, yet that males may follow a successful mating season

with an unsuccessful one. However, any conclusions from these data must be considered tentative given the limited sample size (n = 6) studied over only 2 seasons. Interseasonal data from a larger sample of males over several years would be required to examine the possible effects of previous breeding experience on male reproductive performance and the plasticity of mating tactics and behaviour.

Male haul-out locations, movements and mating patterns

For males which hauled-out relatively regularly at the same location (those with a site fidelity index of less than 1); the average distance from their preferred haul-out site to the last sighted location of the female which he mated was 6.9 ± 1.6 km (n = 10, range 0.9 to 19.6 km); which supports the indication in Chapter IV that females do not necessarily mate with males located in or near their haul-out group. If haul-out locations reflect movement patterns at sea, this would suggest that some females move along the colony and mate with males stationed in home ranges away from their usual haul-out locations. Some males clearly moved between groups more frequently, however, and may do so in search of mates. Without evidence that the movement of males between locations on the beach reflects their movement patterns at sea, it is not possible to differentiate these possibilities. Concurrent with this study, male harbour seals were fitted with radio-transmitters and their locations at sea monitored by telemetry (D.J. Boness and W.D. Bowen, unpublished data) and the information gained in that study may prove useful to test this idea.

Overall, no single measured variable was significantly correlated with any measure of mating success, therefore it would be difficult to accurately predict male mating success from the measured phenotypic characteristics presented in this study. On one hand, this is not surprising since mating success does not vary greatly among males. On another level, mating success is likely to be a multivariate function of many complex behaviours, and a certain degree of chance. Males do vary greatly in their behaviour patterns, and some combinations of the measured phenotypic characteristics (such as wounding and frequently hauling out alone for Group A males) are associated with a low probability of mating success. Other combinations of phenotype, such as large initial body mass and high reproductive effort in the pre-mating period (characteristic of Group B males) were often associated with mating success, but not all successful males were large or showed evidence of competition in the pre-mating period. Larger males, or males in better initial condition, may have greater flexibility in timing their reproductive effort with respect to the appearance of oestrus females, but this does not always predict mating success. If it did, we might expect harbour seals to be more size dimorphic. It is also possible that males may adopt different mating tactics within or between seasons which have different, and probably unmeasured, predictors of mating success. Factors such as previous breeding experience or age, the intensity or frequency of underwater vocalizations (Hanggi and Schusterman 1994) or the movement patterns of males at sea (D.J. Boness and W.D. Bowen unpublished data) may be differentially important to mating success, depending on whether a

male is advertising on a home range or actively searching for females. Both appear to be possible mating tactics for male harbour seals at Sable Island.

CHAPTER VI

GENERAL CONCLUSIONS

Several studies have previously described the combination of energetic costs, activity patterns and mating success of individual males in terrestrially breeding species, but this study is the first to integrate such data in an aquatically mating pinniped. The results of this study indicate that reproduction is as energetically costly for male harbour seals as it is for males of highly dimorphic, terrestrially breeding species. Due to small body size, most male harbour seals cannot energetically afford the extended fast which is characteristic of other breeding pinnipeds, hence they forage during deep diving bouts until a time when most females are likely to become receptive. All males changed their diving behaviour during the mating period to repeated bouts of shallow diving activity, which reflect reproductive behaviour. Males which have greater initial body stores spend less time making deep diving trips to sea, and they expend more energy from their initial body stores to afford to compete for females earlier in the breeding season.

Genetic analysis of paternity in two cohorts of pups indicated that harbour seals are polygynous, as the most successful male harbour seals may sire as many as 6 pups in a season, but the variance in mating success among males was low, with most males likely to have sired one or no pups. This is consistent with the limited ability of males to monopolize access to females in the aquatic

mating environment, and with the widespread distribution of females along the north beach and at sea near Sable Island. There was no evidence to suggest that males mate with females which are located nearby on shore, nor do females appear to exhibit mate fidelity between seasons. Females may mate with males from any part to the colony.

Male mating success could not be reliably predicted from any single phenotypic characteristic, however, multivariate analysis identified suites of characteristics which are associated with varying degrees of reproductive success. Relatively large males which hauled out alone and bore wounds from fighting were reproductively unsuccessful, and probably represent individuals which have been defeated in intrasexual competition. The group of males with the highest rate of mating success tended to have the greatest energy stores at the beginning of the mating season, and they started to compete for mates earlier and at greater energetic expense than other males.

REFERENCES

Allen, P.J., Amos, W., Pomeroy, P.P. and Twiss, S.D. 1996. Microsatellite variation in grey seals (*Halichoerus grypus*) shows evidence of genetic differentiation between two British breeding colonies. Mol. Ecol. In press.

Amos, B., Twiss, S., Pomeroy, P. and Anderson, S. 1995. Evidence for mate fidelity in the grey seal. Science **268**:1897-1899.

Anderson, S. S. and Fedak, M.A. 1985. Grey seal males: energetic and behavioural links between size and sexual success. Anim. Behav. **33**:829-838.

Arnould, J., Boyd, I.L. and Speakman, J.R. 1996. Measuring the body composition of Antarctic fur seals (*Arctocephalus gazella*): validation of hydrogen isotope dilution. Physiol. Zool. **69**:93-116.

Atkinson, S. and Gilmartin, W.G. 1992. Seasonal testosterone pattern in Hawaiian monk seals (*Monachus schauinslandi*). J. Reprod. Fert. **96**:35-39.

Bartholomew, G.A. 1970. A model for the evolution of pinniped polygyny. Evolution **24**:546-559.

Bartholomew, G.A. and Hoel, P.G. 1953. Reproductive behaviour of the Alaska fur seal, *Callorhinus ursinus*. J. Mammal. **34**:417-436.

Bartsh, S.S., Johnston, S.D. and Siniff D.B. 1992. Territorial behaviour and breeding frequency of male Weddell seals (*Leptonychotes weddelli*) in relation to age, size, and concentrations of serum testosterone and cortisol. Can. J. Zool. **70**:680-692.

Bigg, M.A. 1969. The harbour seal in British Columbia. Bull. Fish. Res. Board Can. **172**:1-33.

Boness, D.J. 1984. Activity budgets of male gray seals, *Halichoerus grypus*. J. Mammal. **65**:291-297.

Boness, D.J. 1991. Determinants of mating systems in the Otariidae (Pinnipedia). *In* Behaviour of Pinnipeds. *Edited by* D. Renouf. Chapman and Hall, London. Pp 1-44.

Boness, D.J. and James, H. 1979. Reproductive behaviour of the grey seal (*Halichoerus grypus*) on Sable Island, Nova Scotia. J. Zool. Lond. **188**:477-500.

Boness, D.J., Bowen, W.D. and Oftedal, O.T. 1988. Evidence of polygyny from spatial patterns of hooded seals (*Cystophora cristata*). Can. J. Zool. **66**:703-706.

Boness, D.J., Bowen, W.D. and Francis, J.M. 1993. Implications of DNA fingerprinting for mating systems and reproductive strategies of pinnipeds. Symp. Zool. Soc. Lond. **66**:61-93.

Boness, D.J., Bowen, W.D. and Oftedal, O.T. 1994. Evidence of a maternal foraging cycle resembling that of otariid seals in a small phocid, the harbor seal. Behav. Ecol. Sociobiol. **34**:95-401.

Boulva, J. and McLaren, I.A. 1979. Biology of the harbour seal (*Phoca vitulina*), in eastern Canada. Bull. Fish. Res. Board Can. **200**:1-24.

Bowen, W.D., Oftedal, O.T. and Boness, D.J. 1992. Mass and energy transfer during lactation in a small phocid, the harbor seal (*Phoca vitulina*). Physiol. Zool. **65**:844-866.

Boyd, I.L. 1989. Spatial and temporal distribution of Antarctic fur seals (*Arctocephalus gazella*) on the breeding grounds at Bird Island, South Georgia. Polar Biol. **10**:179-185.

Boyd, I.L. and Duck, C.D. 1991. Mass changes and metabolism in territorial male Antarctic fur seals (*Arctocephalus gazella*). Physiol. Zool. **64**:375-392.

Bradbury, J.W. and Gibson, R.M. 1983. Leks and mate choice. *In* Mate choice. *Edited by* P. Bateson. Cambridge University Press, Cambridge. Pp. 109-138.

Bruford, M.W. and Wayne, R.K. 1993. Microsatellites and their application to popular genetic studies. Curr. Opin. Gen. Develop. **3**:939-943.

Burke, T. 1989. DNA fingerprinting and other methods for the study of mating success. Trends Ecol. Evol. **4**:139-144.

Clutton-Brock, T.H. 1988. Reproductive success. *In* Reproductive success. *Edited by* T.H. Clutton-Brock. University of Chicago Press, Chicago. Pp. 472-487.

Clutton-Brock, T.H. 1989. Mammalian mating systems. Proc. R. Soc. Lond. **36**:339-372.

Coltman, D.W., Bowen, W.D. and Wright, J.M. 1996. PCR primers for harbour seal (*Phoca vitulina concolour*) microsatellites amplify polymorphic loci in several pinniped species. Mol. Ecol. **5**:161-163.

Coltman, D.W. and Wright, J.M. 1994. *Can* SINEs: a family of tRNA-derived retroposons specific to the superfamily Canoidea. Nuc. Acids Res. **22**:2726-2730.

Costa, D. P. and Gentry, R.L. 1986. Free-ranging energetic of northern fur seals. *In* Fur seals: maternal strategies on land and at sea. *Edited by* R.L. Gentry and G. L. Kooyman. Princeton University Press, Princeton. Pp. 79-101.

Costa, D. P., Le Boeuf, B.J. Ortiz, C.L. and Huntley, A.C. 1986. The energetics of lactation in the northern elephant seal. J. Zool. Lond. **209**:21-33.

Costa, D. P., Croxall, J.P. and Duck, C.D. 1989. Foraging energetics of Antarctic fur seals in relation to changes in prey availability. Ecology **70**:596-606.

Craighead, L, Paetkau, D., Reynolds, H.V, Vyse, E.R. and Strobeck, C. 1995. Microsatellite analysis of paternity and reproduction in Arctic grizzly bears. J. Heredity **86**:255-261.

Creel, S., Wildt, D.E and Monfort, S.L. 1993. Aggression, reproduction, and androgens in wild dwarf mongooses: a test of the challenge hypothesis. Am. Nat. **141**:816-825.

Crews, D. and Moore, M.C. 1986. Evolution of mechanisms controlling mating behaviour. Science **231**:121-125.

Darwin, C. 1859. The origin of species. Murray, London.

Darwin, C. 1871. The descent of man and selection in relation to sex. Murray, London.

Davies, N.B. 1991. Mating systems. *In* Behavioural ecology (3rd edn.). *Edited by* J.R. Krebs and N.B. Davies. Blackwell Scientific, London. Pp. 263-294.

Davis, M.B. and Renouf, D. 1987. Social behaviour of harbour seals, *Phoca vitulina*, on haulout grounds at Miquelon. Can. Field Nat. **101**:1-5.

Deutsch, C.J., Haley, M.P. and Le Boeuf, B.J. 1990. Reproductive effort of male northern elephant seals: estimates from mass loss. Can. J. Zool. **68**:2580-2593.

Devlin, B., Roeder, K. and Ellstrand, N.C. 1988. Fractional paternity assignment: theoretical development and comparison to other methods. Theor. Appl. Genet. **76**:369-380.

Duffy, A.J., Coltman, D.W. and J.M. Wright. 1996. Microsatellites at a common site in the second ORF of L1 elements in mammalian genomes. Mamm. Genome **7**:386-387.

Emlen, S.T. and Oring, L.W. 1977. Ecology, sexual selection and the evolution of mating systems. Science **197**:215-223.

Estoup, A., Scholl, A., Pouvreau, A. and Solignac, M. 1995. Monoandry and polyandry in bumble bees (Hymenoptera; Bombinae) as evidenced by highly variable microsatellites. Mol. Ecol. **4**:89-93.

Everitt, B.E. 1974. Cluster analysis. Heinemann Educational Books, London.

Fedak, M. A. and Anderson, S.S. 1982. The energetics of lactation: accurate measurements from a large wild mammal, the grey seal (*Halichoerus grypus*). J. Zool. Lond. **198**:473-479.

Fedak, M. A. and Anderson, S.S. 1987. Estimating the energy requirements of seals from weight changes. *In* Marine mammal energetics. *Edited by* A. C. Huntley, D. P. Costa, G. A. J. Worhty and M. A. Castellini. Society for marine mammalogy special publication no. 1. Pp. 205-223.

Francis, J. and Boness, D.J. 1991. The effect of thermoregulatory behaviour on the mating system of the Juan Fernandez fur seal, *Arctocephalus phillippii*. Behaviour **199**:104-127.

Godsell, J. 1988. Herd formation and haul-out behaviour in harbour seals (*Phoca vitulina*). J. Zool. Lond. **215**:83-98.

Goode, B. L., and Feinstein, C. 1992. Speedprep purification of template for double stranded DNA sequencing. Biotechniques **12**:374-375.

Hanggi, E.B. and Schusterman, R.J. 1994. Underwater acoustic displays and individual variation in male harbour seals, *Phoca vitulina*. Anim. Behav. 48:1275-1283.

Harding, C.F. 1981. Social modulation of circulating hormone levels in the male. Amer. Zool. **24**:223-231.

Harris, A.S., Young, J.S. and Wright, J.M. 1991. DNA fingerprinting of harbour seals (*Phoca vitulina concolour*): male mating behaviour may not be a reliable indicator of reproductive success. Can. J. Zool. **69**:1862-1866.

Hill. S.E.B. 1987. Reproductive ecology of Weddell seals (*Leptonychotes weddelli*) in McMurdo Sound, Antarctica. PhD. Thesis, University of Minnesota.

Iverson, S.J., Bowen, W.D. Boness, D.J. and Oftedal, O.T. 1993. The effect of maternal size and maternal milk energy output on pup growth in grey seals (*Halichoerus grypus*). Physiol. Zool. **66**:61-88.

Inoue, M., Mitsunaga, F., Ohsawa, H., Takenaka, A., Sugiyama, Y., Gaspard, S.A. and Takenaka, O. 1991. Male mating behaviour and paternity discrimination by DNA fingerprinting in a Japanese macaque group. Folia Primatol. **56**:202-210.

Jain, A.K. and Dubes, R.C. 1988. Algorithms for clustering data. Prentice Hall, New Jersey.

Jeffreys, A.J., Wilson, V., and S. L. Thein. 1985a. Hypervariable 'minisatellite' regions in human DNA. Nature **314**:67-73.

Jeffreys A.J., Wilson, V. and S.L. Thein. 1985b. Individual-specific 'fingerprints' of human DNA. Nature **316**:76-79.

Keiver, Ronald, K. and Beamish, F. 1984. Metabolizable energy requirements for maintenance and faecal and urinary losses of juvenile harp *seals (Phoca groenlandica*). Can. J. Zool. **62**:769-776.

Kleiber, M. 1975. The fire of life: an introduction to animal energetics. R. E. Kreiger Publishing Company, Huntington, NY.

Kleiman, D.G. 1977. Monogamy in mammals. Q. Rev. Biol. **52**:39-69.

Kovacs, K.M. 1990. Mating strategies in male hooded seals *(Cystophora cristata*)? Can. J. Zool. **68**:2499-2502.

Lavigne, D.M., Worthy, G.A.J., Kovacs, K.M., Schmidt, O.J. and Hickie, J.P. 1987. Metabolic rates of seals and whales. Can. J. Zool. **64**:279-284.

Le Boeuf, B.J. 1974. Male-male competition and reproductive success in elephant seals. Am. Zool. **14**:163-176.

Le Boeuf, B.J. 1991. Pinniped mating systems on land, ice, and in the water: emphasis on the Phocidae. *In* Behaviour of Pinnipeds. *Edited by* D. Renouf. Chapman and Hall, London. Pp. 45-65.

Le Boeuf, B.J. and Reiter, J. 1988. Lifetime reproductive success in male and female northern elephant seals. *In* Reproductive success. *Edited by* T.H. Clutton-Brock. University of Chicago Press, Chicago. Pp. 344-362.

Lewin R (1989) Limits to DNA fingerprinting. Science **243**:1549-1551.

Levinson, G. and Gutman, G.A. 1987. Slipped-strand mispairing: a major mechanism for DNA sequence evolution. Mol. Biol. Evol. **4**:203-221.

Lincoln, G.A. 1981. Seasonal aspects of testicular function. In The testis. Edited by H. Burger and D deKrester. Raven Press, New York. Pp. 255-302.

Lindstedt, S. L. and Boyce, M.S. 1985. Seasonality, fasting endurance, and body size in mammals. Am. Nat. **125**:873-878.

Litt, M. and Luty, J.A. 1989. A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am. J. Human Gen. **48**:397-401.

Lydersen, C., Hammill, M.O. and Ryg, M.S. 1992. Water flux and mass gain during lactation in free-living ringed seal (*Phoca hispida*) pups. J. Zool. Lond. **228**:361-369.

Markussen, N. H. and Ryg, M. 1992. Metabolic rate and body composition of harbour seals, *Phoca vitulina*, during starvation and refeeding. Can. J. Zool. **70**:220-224.

McCann, T.S. 1981. Aggression and sexual activity of male Southern elephant seals, *Mirounga leonina*. J. Zool. Lond. **195**:295-310.

McLaren, I.A. 1993. Growth in pinnipeds. Biol. Rev. **68**:1-79.

Meagher, T.R. 1986. Analysis of paternity within a natural population of *Chamaelirium luteum*. 1. Identification of most-likely male parents. Am Nat. **128**:199-215.

Milligan, G.W. 1980. An examination of the effects of six types of error perturbation on fifteen clustering algorithms. Psychometrika **45**:325-342.

Minitab reference manual. 1994. Minitab release 10 for Windows. Minitab Inc, State College, Pa.

Mödig, A.O. 1996. Effects of body size and harem size on male reproductive behaviour in the southern elephant seal. Anim Behav. **51**:1295-1306.

Moore, M.C. 1987. Circulating steroid hormones during rapid aggressive responses of territorial male mountain spiny lizards, *Sceloporus jarrovi*, Horm. Behav. **21**:511-521.

Morin, P.A., Wallis, J., Moore, J.J. and Woodruff, D.S. 1994. Paternity exclusion in a community of wild chimpanzees using hypervariable simple sequence repeats. Mol. Ecol. **3**:469-478.

Muelbert, M.M. and Bowen, W.D. 1993. Duration of lactation and postweaning changes in mass and body composition of harbour seal, *Phoca vitulina*, pups. Can. J. Zool. **71**:1405-1414.

Murray Jr., B.G. 1992. The evolutionary significance of lifetime reproductive success. The Auk **109**:167-172.

Nagy, K. A. 1987. Field metabolic rate and food requirement scaling in mammals and birds. Ecol. Monog. **57**:111-128.

Noonan, L.M., Ronald, K. and Raeside, J. 1991. Plasma testosterone concentrations of captive male hooded seals (*Cystophora cristata*). Can. J. Zool. **69**:2279-2282.

Oftedal, O. T., Boness, D.J. and Bowen, W.D. 1993. Energy transfer by lactating hooded seals and nutrient deposition in their pups during the four days from birth to weaning. Physiol. Zool. **66**:412-436.

Oftedal, O. T. and Iverson, S.J. 1987. Hydrogen isotope methodology for measurement of milk intakes and energetics of growth in suckling young. *In* Marine mammal energetics. *Edited by* A. C. Huntley, D. P. Costa, G. A. Worthy and M. A. Castellini. Society for marine mammalogy special publication no. 1. Pp. 67-89.

O'Reilly, P. and Wright, J.M. 1995. The evolving technology of DNA fingerprinting and its application to fisheries and aquaculture. J. Fish Biol. **47** (Suppl. A):29-55.

Orians, G.H. 1969. On the evolution of mating systems in birds and mammals. Am. Nat. **103**:589-603.

Pace, N. and Rathbun, 1945. Studies on body composition. III. The body water and chemically combined nitrogen content in relation to fat content. J. Biol. Chem. **158**:685-691.

Pemberton, J.M., Albon, S.D., Guiness, F.E. Clutton-Brock, T.H. and Dover, G.A. 1992. Behavioral estimates of male mating success tested by DNA fingerprinting in a polygynous mammal. Behav. Ecol. **3**:66-75.

Pemberton, J.M., Slate, J., Bancroft., and Barrett, J.A. 1995. Non-amplifying alleles at microsatellite loci: a caution for parentage and population studies. Mol. Ecol. In press.

Perry, E.A. 1993. Aquatic territory defence by male harbour seals (*Phoca vitulina*) at Miquelon: relationship between active defence and male reproductive success. PhD. Thesis. Memorial University of Newfoundland, St. John's.

Peters, H.R. 1983. The ecological implications of body size. Cambridge University Press, Cambridge.

Rayner, J.M.V. Linear relations in biomechanics: the statistics of scaling functions. J. Zool. Lond. **206**:415-439.

Reijnders, P.J.H. 1990. Progesterone and oestradiol-17B concentration profiles throughout the reproductive cycle in harbor seals (*Phoca vitulina*). J. Reprod. Fert. **90**:403-409.

Reilly, J. J. and Fedak, M.A. 1990. Measurement of the body composition of living grey seals by hydrogen isotope dilution. J. Appl. Physiol. **69**:885-891.

Reilly, J. J. and Fedak, M.A. 1991. Rates of water turnover and energy expenditure of free-living male common seals (*Phoca vitulina*). J. Zool. Lond. **223**:461-468.

Richard, K.R, Whitehead, H. and Wright, J.M. 1996. Polymorphic microsatellites from sperm whales and their use in genetic identification of individuals from naturally sloughed pieces of skin. Mol. Ecol. **5**:313-315.

Ronald, K., Keiver, K.M., Beamish, W.H. and Frank, R. 1984. Energy requirements and faecal and urinary losses of the grey seal (*Halichoerus grypus*). Can. J. Zool. **62**:1101-1105.

Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B. and Erlich, H.A. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239**:487-491.

Sambrook, J., Fritsh, E.F. and Maniatis, T. 1989. Molecular cloning: a laboratory manual. 2nd edn. Cold Spring Harbour Press, New York.

Schmidt-Nielson, K. 1979. Animal physiology, adaptations and environment. Cambridge University Press, Cambridge.

Siniff, D.B., Stirling, I., Bengtson, J.L and Reichle, R.A. 1979. Social and reproductive behaviour of crabeater seals (*Lobodon carcinophagus*) during the austral spring. Can. J. Zool. **57**:2243-2255.

SPSS. 1995. Statistical package for the social sciences for Windows (version 6.1.3). SPSS Inc., USA.

Stirling, I. A. 1983. The evolution of mating systems in pinnipeds. *In*: Advances in the study of mammalian behaviour. *Edited by* J. F. Eisenberg and D. G. Kleiman. American Society of Mammalogists, Shippensburg, Pa. Pp. 489-527.

Stoddart, D.M., Bradley, A.J. and Mallick, J. 1994. Plasma testosterone concentration, body weight, social dominance and scent-marking in male marsupial sugar gliders (*Petaurus breviceps*; Marsupialia: PetauridaeZ). J. Zool. Lond. **232**:595-601.

Sullivan, R.M. 1981. Aquatic displays and interaction in the harbour seal, *Phoca vitulina*, with comments on mating systems. J. Mammal. **62**:825-831.

Sullivan, R.M. 1982. Agonistic behaviour and dominance relationships in the harbour seal (*Phoca vitulina*). J. Mammal. **63**:554-569.

Takenaka O., Kawamoto, S., Udono, T., Arakawa, M., Takasaki, H. and Takenaka, A. 1993a. Chimpanzee microsatellite PCR primers applied to paternity testing in a captive colony. Primates **34**:357-363.

Takenaka, O., Takasaki, H., Kawamoto, S., Arakawa, M. and Takenaka, A. 1993b. Polymorphic microsatellite DNA amplification customized for chimpanzee paternity testing. Primates **34**:27-35.

Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nuc. Acids Res. **17**:6463-6471.

Tautz, D. 1990. Genomic fingerprint goes simple. Bioessays **12**:44-46.

Thompson, E.A. 1976. Inference of genealogical structure. Soc. Sci Inform. **15**:477-526.

Thompson, P.M. 1988. Timing of mating in the common seal (*Phoca vitulina*). Mamm. Rev. **18**:105-112.

Thompson, P. M., Fedak M.A., McConnell, B.J and Nicholas, K.S. 1989. Seasonal and sex-related variation in the activity patterns of common seals (*Phoca vitulina*). J. Appl. Ecol. **26**:521-535.

Tinker, M.T., Kovacs, K.M. and Hammill, M.O. 1995. The reproductive behavior and energetics of male gray seals (*Halichoerus grypus*) breeding on a land-fast ice substrate. Behav. Ecol. Sociobiol. **36**:159-170.

Trivers, R. L. 1972. Parental investment and sexual selection. *In* Sexual selection and the descent of man, 1871-1971. *Edited by* B. Campbell. Aldine, Chicago. Pp.136-179.

Van Parijs, S.M, Thompson, P.M., Tollit, D.J. and MacKay, A. 1996. Changes in the distribution and activity of male harbour seals during the breeding season. Anim. Behav. In press.

Wade, M.J. and Arnold, S.J. 1980. The intensity of sexual selection in relation to male behaviour, female choice, and sperm precedence. Anim. Behav. **28**:446-461.

Walker, B.G. and Bowen, W.D. 1993a. Changes in body mass and feeding behaviour in male harbour seals, *Phoca vitulina*, in relation to female reproductive status. J. Zool. Lond. **231**:423-436.

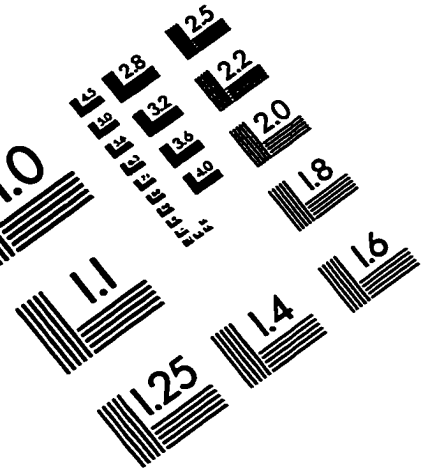
Walker, B.G., and Bowen, W.D. 1993b. Behavioural differences among adult male harbour seals during the breeding season may provide evidence of reproductive strategies. Can. J. Zool. **71**:1585-1591.

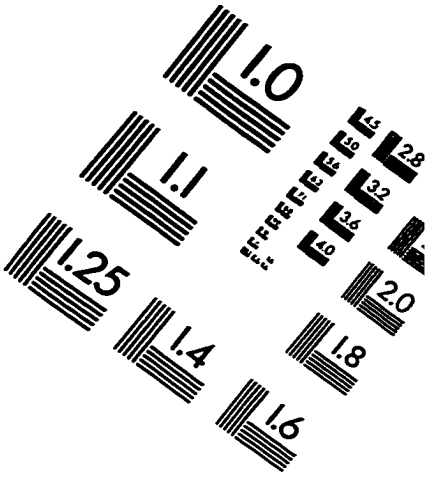
Weber, J.L. and May, P.E. 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am. J. Human Gen. **44**:388-396.

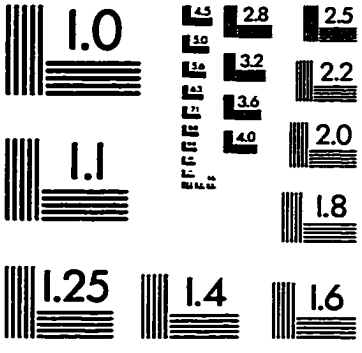
Wingfield, J.C., Hegner, R.E., Dufty, A.M. and Ball, G.F. 1990. The "challenge hypothesis": theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. **136**:829-846.

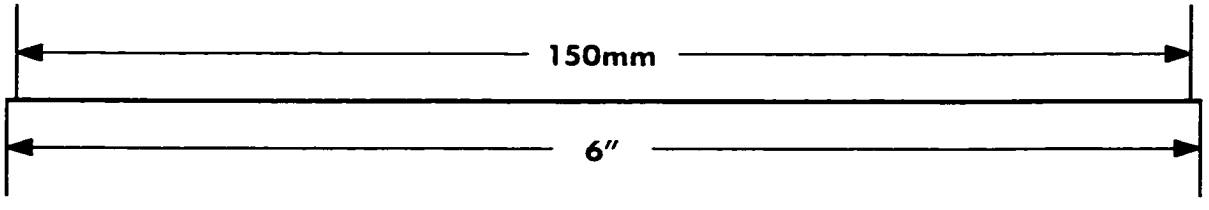
Zielinski, W.J. and Vandenbergh, J.G. 1993. Testosterone and competitive ability in male house mice, *Mus musculus*: laboratory and field studies. Anim. Behav. **45**:873-891.

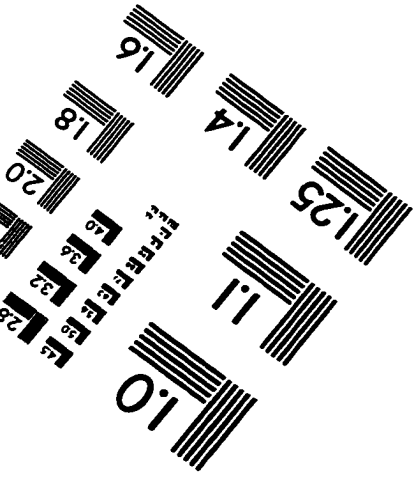
IMAGE EVALUATION TEST TARGET (QA-3)

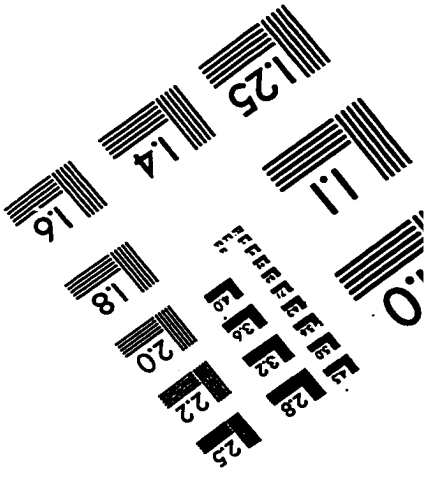












APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989