POSSIBLE EFFECTS OF POLLOCK AND HERRING ON THE GROWTH AND REPRODUCTIVE SUCCESS OF STELLER SEA LIONS: INSIGHTS FROM FEEDING EXPERIMENTS USING AN ALTERNATIVE ANIMAL MODEL, *RATTUS NORVEGICUS*

by

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ABSTRACT

The decline of Steller sea lions (*Eumetopias jubatus*) in the Gulf of Alaska appears to have been associated with a switch of diet from one dominated by fatty forage fishes (such as herring - *Clupea pallasi*) to one dominated by low fat fish (such as pollock - *Theragra chalcogramma*). Observations made during the decline include reduced body size of sea lions, low pregnancy rates, poor fur quality and high mortality. I used the general mammalian model, *Rattus norvegicus*, to test whether changes in size and reproductive performance could be caused by a switch in the quality of prey consumed. I fed five groups of 12 female, weanling rats diets composed of herring (H), pollock (P), pollock supplemented with herring oil (PH), pollock supplemented with pollock oil (PP), or a semi-purified diet (ICN). Mean body weights were greatest for H, followed by PH, P, PP and finally ICN, although ICN was the only group significantly different from the others. Food intakes prior to mating were 10% higher for groups on the lower fat diets (P and ICN), resulting in similar caloric intakes in all groups. Efficiency of energy utilization was also similar for all fish diets. However, this efficiency was slightly reduced when pollock was supplemented with oil (PP and PH) compared to pollock alone. The protein efficiency ratio (PER) was highest for the H diet, slightly lower for all pollock diets, and significantly lower for ICN.

Rats fed the low energy P and ICN meals did not compensate by consuming more during gestation. The fetal weights for mothers fed pollock (P) were significantly reduced. This study shows that the caloric content was a major limiting factor in the nutritional quality of pollock. If food intake was adjusted to meet energetic requirements, there were no detrimental consequences to eating pollock. However, supplementation of

pollock meal with additional pollock oil may reduce growth and reproductive performance, although the reasons for this were not apparent.

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CHAPTER I : The Nutritional Stress Hypothesis and the Decline and Prey of Steller Sea Lions in Alaska

Introduction

Kenyon and Rice (1961) estimated that Gulf of Alaska (GOA) and the Aleutian Islands (AI) were home to approximately 183 000 Steller sea lions (*Eumetopias jubatus*) in the late 1950s, while the worldwide population at this time was estimated at over 300 000 Steller sea lions (Loughlin 1998). Subsequent surveys showed a significant decline in abundance with only 112 000 in the GOA and AI in 1989 (Trites and Larkin 1996) and as few as 116 000 worldwide (Loughlin *et al.* 1992).

The decline in Steller sea lion abundance did not happen all at once, nor evenly throughout the range of the species. Trites and Larkin (1996) estimate that the decline commenced in the eastern Aleutians in the mid 1960s and spread throughout the Aleutians, the Bering Sea and the Gulf of Alaska. By 1979, all regions were suffering, with the exception of southeastern Alaska. By 1989, the Steller sea lion population was only one-third of its 1950's size (Loughlin 1998) and in the western portion of their range, only 10-20% of the population endured (Calkins *et al.* 1998).

What provoked this devastating decline? A recent review by Loughlin (1998) offered ten possible mechanisms to the Steller sea lion population decline: redistribution, pollution, predation, subsistence use, commercial harvest, disease, natural fluctuation, environmental changes, changed vital rates and commercial fishing. Within this review, the first seven explanations were deemed unlikely to have caused the catastrophic decline

in sea lion population size. Trites (1998), after reviewing fisheries data, concluded that it was unlikely that fisheries caused the decline through over-exploitation of sea lion prey or entanglement in nets.

The last two possibilities offered in Loughlin's review, environmental changes and changed vital rates (e.g. birth or death rate), could be interrelated. Known environmental fluctuations, such as a slight increase in water temperature in the Bering Sea in the 1970s, probably caused shifts in the abundance of various species of fish during the last 30 years (Anderson *et al.* 1997; Springer 1998; Schumacher and Alexander 1999). This could affect vital rates of Steller sea lions through malnutrition caused by an inadequate quantity, quality or accessibility of prey. It is this "nutritional stress" hypothesis that is now considered by many to be the most likely cause of the Steller sea lion population decline (Calkins and Goodwin 1988; Merrick and Calkins 1996; Calkins *et al.* 1998; Trites 1998).

A Switch in Prey

The nutritional stress hypothesis is based on the assumption that the diet of the Steller sea lions changed prior to and during the period of population decline.

From 1975 to 1978, Pitcher (1981) collected the stomach contents of over 250 sea lions in the Gulf of Alaska and compared his results to collections taken between 1958 and 1960. The primary difference between the pre- and post-decline eras was that walleye pollock *(Theragra chalcogramma)* was the dominant prey species in later samples, but was largely absent in the earlier collections. Pitcher pointed out that walleye pollock stocks increased by 40% of the total demersal fish stocks concurrent with the

Table 1. Proportion of Steller sea lion stomachs and scats containing seven prey categories for summer Kodiak Island area collections made during 1976-1978, 1985-1986, and 1990-1993 (from Merrick *et al.* 1997).

Period	Sample Size	Gadids (%)	Salmon (%)	Small schooling (%)	Flat-fish (%)	Other (%)	Atka mackerel (%)	Squid or octopus (%)
1990-1993	54	85.2	18.5	18.5	13.0	0.0	0.0	11.1
1985-1986	20	60.0	5.0	20.0	5.0	0.0	0.0	20.0
1976-1978	28	32.1	17.9	60.7	0.0	0.0	0.0	0.0

Steller sea lion decline in the Gulf of Alaska. A later study by Merrick *et al.* (1997) showed that the dominant food source of Steller sea lions from 1976-1978 was small schooling fish (primarily herring, *Clupea pallasi*), occuring in 61% of sea lion scats and stomachs (Table 1). By 1990-1993, however, the proportion of collections containing small schooling fishes fell to 18.5% while pollock and other gadids rose to over 85%.

Merrick (1997) demonstrated that sea lion populations seemed to decrease most dramatically in areas where diet was the least diverse and dominated by pollock or Atka mackerel. Populations that have been increasing in southeast Alaska have a very diverse diet (Trites, unpublished data).

Evidence for a diet switch concurrent with the decline in Steller sea lion abundance is extensive, but correlations are not necessarily causal. The nutritional stress hypothesis, however, is based on the apparent dietary change in dominant prey *in combination* with observations of morphological alterations in pre- and post- decline animals.

Observations of Nutritional Stress in Steller Sea Lions

Typical symptoms for mammals under nutritional stress include reduced growth and weight, poor fur/hair quality, prolonged adolescent infertility, reduced overall fertility and high mortalities (Calkins *et al.* 1998; Guinet *et al.* 1998; Trites 1998). Observations of wild Steller sea lions in the 1980's and 1990's noted many of these symptoms including reduced body size as compared to pre-decline sizes and reduced reproductive success (Calkins *et al.* 1998; Trites 1998).

Reduced Body Size

The results of two population surveys provide strong evidence for a population-wide reduction in Steller sea lion body size when comparing pre- and post- decline animals.

Steller sea lion body weights, standard lengths and axillary girths were all significantly smaller in animals aged 1 to10 years during the population decline compared to measurements taken prior to the decline (Calkins and Goodwin 1988). These changes were independent of reproductive status. In 1985-1986, sea lions 1 to 5 years old were 4.5% shorter and 22.5% lighter than those in the 1970s, and animals 6 to 10 years of age were 1.2% shorter and 6.9% lighter than pre-decline animals (Lowry *et al.* 1988).

Theories regarding density-dependent responses predict that body size should have increased as population density decreased, because the amount of food available to

Table 2. The estimated reductions in axillary girth and weight in Steller seal lions aged 1, 7 and 14 years in 1985-1986 (population decline) as compared to 1975-1978 (pre-decline) (from Calkins *et al.*, 1998).

	% Reduction in axillary girth	% Reduction in weight
Age 1	10.4	26.9
Age 7	6.29	12.3
Age 14	1.7	3.0

each individual would have increased with a reduction in sea lion abundance (Calkins and Goodwin 1988). The fact that body size decreased during this decline suggests that there was a decrease in per capita food abundance (Calkins and Goodwin 1988). Such a response further suggests that the carrying capacity for Steller sea lions may be lower now than it was in the past.

The Calkins and Goodwin (1988) data set of body size measurements was re-examined in 1993 by Castellini and Calkins (1993). They corroborated the conclusion that sea lions were shorter, lighter and thinner in the 1980s than in the 1970s. However, they also noted that the sea lions had less body fat and/or a different body shape as determined by body volume/weight relationships. Such observations are expected for animals that are nutritionally stressed. Furthermore, this reduction in body size seemed to be more apparent in juvenile sea lions than in adults, implying slower growth of the most recent generations due to limited food resources (Sease and Merrick 1997). Further use of multiple regression models on length, girth and weight measurements supported the notion that the greatest reductions in size were in the youngest animals (Calkins *et al.* 1998) (Table 2).

Body length is believed to reflect nutritional status during the first 8 to 9 years of life, while weight and girth likely reflect recent nutritional condition, in addition to lifetime nutrition (Calkins and Pitcher 1982). It appears that younger animals (Table 2) were more nutritionally stressed than adults at the time of measurement, as seen by the larger reductions in their weights and girths as compared to the older animals. Due to similar lengths in pre- versus post-decline era animals (23.12 cm versus 23.20 cm), less adequate nutrition apparently affected the adults in this study later in life, versus during their weaning and juvenile years.

Reduced Productivity

Reduced fertility is a well known outcome of nutritional stress in mammals and involves complex interactions between the metabolic and reproductive pathways (Kirkwood and Aherne 1985; Bronson 1988; Booth 1990; Pharazyn *et al.* 1991; Newton and Mahan 1992; Den Hartog and Vesseur 1993; Rozeboom *et al.* 1993; Coffey *et al.* 1994; Barash *et al.* 1996; O'Dowd *et al.* 1997; Shaw *et al.* 1997; Amico *et al.* 1998; Aubert *et al.* 1998; Barb *et al.* 1998; Keisler *et al.* 1998; Brann *et al.* 1999; Bulik *et al.* 1999; Cunningham *et al.* 1999; Nieuwenhuizen *et al.* 1999; Pinilla *et al.* 1999). One of the repercussions of inadequate nutrition can be delayed first estrus in juveniles resulting in delayed age at first reproduction (Wilen and Naftolin 1978; Frisch *et al.* 1980; Bronson 1988; Beltranena *et al.* 1991). It can also lengthen the interval from parturition to the following estrus, and can result in low birth weights, spontaneous abortions, and low survival of newborns (Bulik *et al.* 1999).

Fertility parameters such as delayed first estrus, anestrus (temporary cessation of estrous cycles) and pregnancy rates prior to parturition can be difficult to measure in wild sea lions, particularly if minimal disturbance to these endangered animals is sought. Although measures of fecundity have been estimated in the past, the most common measures of fertility in these populations are pup counts, pup size and growth rates.

Pups remain on or near rookery beaches during the early nursing period, making them easy to count and providing a useful index of annual reproductive success (Calkins and Pitcher 1982). In surveys of Steller sea lions conducted in 1975-1978 and 1985-1986, investigators counted 45% fewer pups in the 1980's than in the 1970's (Calkins and Pitcher 1982; Calkins and Goodwin 1988).

Although pregnancy rates and pup counts provide valuable information about the status of marine mammal populations, they may not be the most complete indicators of population health. Pup birth weights and growth rates are useful indicators of reproductive success as they reflect the mothers' condition during pregnancy, and how much nourishment the pup is receiving through nursing. Unfortunately, data are insufficient to conclude whether or not pup birth weights were lower during the Steller sea lion decline. Nor is there data to compare pup growth rates during the 1970s and 1980s.

Evidence for Nutritional Stress In Controlled Experiments

Analyses of pollock and herring samples led to a refined version of the nutritional stress hypothesis, called the "junk food hypothesis". This specifically states that pollock are an inferior prey source to herring for the Steller sea lion, and that the consumption of

pollock is responsible for the observed signs of nutritional stress in the Steller sea lions, rather than a shortage of fish per se.

Table 3. Proximate composition of Pacific herring and Walleye pollock from Alaska (from Stansby 1976).

	Average % Moisture	Average % Oil	Average % Protein	Average % Ash
Pacific Herring	70.8	12.8	16.4	2.4
Pacific Pollock	81.5	0.98	18.9	1.34

Pacific Herring (*Clupea pallasi*) and Walleye Pollock (*Theragra chalcogramma*)

Pacific herring are small schooling fish that have long supported both fisheries and a variety of marine mammals and birds. They reach a maximum of 46.0 cm and travel in large, pelagic schools (Luna 1999). Herring are seasonal feeders that build up large fat reserves to overwinter (Paul *et al.* 1998) and are classified as a "fatty" fish, with 5-20% fat and 15-20% protein (Stansby 1969).

Herring are the primary fish fed to marine mammals in aquariums such as the Vancouver Aquarium. Fat and protein from herring have also been shown to support growth of study animals such as rats, beyond that of traditional sources of protein and oil such as casein, beef and vegetable oils (Nilson *et al.* 1947; Privett *et al.* 1960).

Walleye pollock are much larger than herring and are also extensively exploited by fisheries, marine mammals and birds. Pollock are demersal fish that can grow to 91.0 cm and weigh up to 1,400 g (Luna 1999). They feed continuously throughout the year, and have relatively stable body compositions. They are classified as a "non-fatty" fish, with less than 5% fat and 15-20% protein (Stansby 1969).

Table 3 is a proximate composition analysis of Pacific herring and walleye pollock (Stansby 1976).

A major difference between the two species of fish is the fat content, which is also the primary factor determining energy density (calories/gram) in fish (Anthony and Roby 1997). Protein content, on the other hand, seems to vary little between the species. The difference in crude fat content may make pollock an inferior prey type. However, there may also be a difference in the "quality" of the fat or proteins that could cause malnourishment in predators.

Laboratory Studies

Physiological and nutritional studies using mammalian models have often incorporated fish proteins and oils into diets to test their nutritional quality. Nilson *et al.* (1947) studied the nutritional value of protein from 18 species of fish, and found that all species were almost equally valuable in promoting growth in rats at a 9% level in the diet. Stansby (1976) also stated that protein contents across fish species are fairly constant and well balanced with respect to essential amino acids. These studies imply that a nutritional difference between pollock and herring may not be due to differences in protein quality.

Privett *et al.* (1960) fed rats diets containing menhaden, tuna, or herring oil and found no significant difference in growth over 85 days between all fish oils. However, their study did not include a gadid species, such as pollock. The fatty acid contents of fish oils are relatively similar with respect to high levels of long-chain, unsaturated fatty acids and the presence of essential fatty acids (Stansby 1969). However, there can be immense

differences in oil compositions between species, and even within species collected from different sites, at different times of the year and at different ages (Stansby 1969). From this evidence, I cannot reject the idea that the nutritional values of different species of fish may be partly attributed to differences in the quality of the fish oils.

Pollock and other gadids are undoubtedly lean fish, but additional qualities also render them less nutritious for pinnipeds. When juvenile harp seals (*Phoca groenlandica*) were switched from a diet of Atlantic herring (*Clupea hargenus*) to Atlantic pollock (*Pollachus virens*), the seals' body fat content declined by 32% over 30 days, while their body protein content increased in proportion to protein intake (Kirsch *et al.* 2000). This loss of body fat occurred despite normal food intakes of approximately 6.5 kg/d, and reflected a change in body composition (reduced fat and increased protein) versus body mass. This implies that marine mammals may not be able to maintain energy reserves on pollock diets despite large intakes. Reductions in body fat caused by eating large amounts of pollock could be detrimental to animals residing in cold environments and subject to periodic fasts between foraging bouts. It could also be detrimental to nursing mothers that require fat stores to produce milk for their young. Concurrent increases in body protein content could be energetically expensive, as protein is metabolically active and increases an animal's caloric requirements.

When switched from diets of herring to pollock, six young captive Steller sea lions lost approximately 0.6 kg/day during short periods of 11-23 days (Rosen and Trites 2000b). Despite the fact that these animals were allowed to eat as much pollock as they wanted, they did not increase their energetic intake sufficiently to compensate for the low caloric content of pollock. Failure to increase their food intake was puzzling and might

be related to factors that determine satiation. Although these animals were able to eat as much as they wanted at feeding times, it is possible that the volume of food required to meet their energetic needs may have been too large to eat in two or three meals. More regular, unlimited feeding might have resulted in increased food intake. A similar result was found when feeding these sea lions low-fat squid. Their intake was approximately 7 kg/d, regardless of diet and nutritional quality, again indicating satiation as a factor controlling food intake (Rosen and Trites 1999). As expected, the sea lions' loss in body weight was accompanied by suppression of mass specific resting metabolic rate, indicating a fasting or nutritionally stressed state.

Earlier studies concluded that the energy required to digest a meal of pollock (the heat increment of feeding, HIF) was higher than that required to digest a similar size meal of herring (15.7% versus 11.9% of gross energy intake, respectively) (Rosen and Trites 1997). Additionally, they found that larger meals were more energetically expensive to consume than smaller meals, which would be costly to an animal attempting to eat more food to compensate for low fat content. The loss of body mass in Steller sea lions eating pollock is therefore attributable to many factors including the lower energy content of pollock, a higher HIF of pollock than herring, and the need to compensate for low energy values of pollock. This means that a Steller sea lion would have to consume 35-80% more pollock than herring to obtain an equal number of calories (Rosen and Trites 2000b).

Negative responses to switching diets from high-energy forage fish (clupeids such as herring and sprat – *Sprattus sprattus*) to gadids have also been documented in wild harbor seals in Scotland (Thompson *et al.* 1997). Blood leukocyte counts were

significantly elevated during years when herring and sprat occurrence in the diet was low (1989-1992) compared to years when they were the major dietary species (1987-1989 & 1993-1995). This could be due to immuno-suppression because of differences in prey nutrient or contaminant levels, or it could be due to differences in water quality between years and between foraging sites. More notably, when the seals switched to a primarily gadid diet, there was evidence of widespread macrocytic anemia thought to be related to differences in the nutritional quality of the prey. Iron levels in white flesh fish such as gadids are lower than in darker flesh fish (Geraci 1975), which might explain the anemic conditions.

Complications in iron absorption have been reported in mink that were fed gadids such as hake and whiting. This results in a condition known as "cotton-fur". This syndrome is characterized by animals that are often emaciated and smaller than their non-cotton fur counterparts, and whose underfur is uncharacteristically light in color due to lack of pigmentation (Stout *et al.* 1960). In one study, inclusion of hake and whiting in mink diets resulted in reduced size of animals and fur depigmentation proportional to the level of inclusion of gadids in the diet (Stout *et al.* 1960). Similarly, mink that were fed diets including 30% hake suffered from lower lifetime weight gain and possible impaired iron absorption as indicated by lower levels of stored iron in the spleen and liver (Rouvinen *et al.* 1997).

Caution should be used when extrapolating results of iron deficiency and poor fur quality to wild animals. Only one of the above studies was conducted in the wild. Furthermore, impaired iron absorption in the captive studies may be a function of the hake having been frozen. When hake were either cooked or fed fresh and unfrozen, this

cotton-fur disorder was less apparent if present at all (Costley 1970). The factor causing cotton-fur is hypothesized to be formaldehyde, a secondary by-product of lipid oxidation, and not present in fresh fish nor applicable to feeding in the wild.

Factors other than body composition and vitamin and mineral content can affect the nutritional value of a diet. The digestive efficiency of diets may reflect "biologically available" nutrition. Juvenile Steller sea lions fed different diets of pollock, herring, squid and salmon revealed differences in digestive efficiency (percentage of prey energy retained) (Rosen and Trites 2000a). The digestive efficiency appeared to have some relationship with energy density, being greatest for herring (95.4%), then pollock (93.9%) and salmon (93.4%), and finally squid (90.4%).

Captive harp seals fed diets of Atlantic cod (*Gadus morhua*), Arctic cod (*Boreogadus saida*), Greenland halibut (*Reinhardtius hippoglossoides*), Atlantic herring (*Clupea harengus*) and capelin (*Mallotus villosus*) showed the lowest digestive efficiencies while consuming the low-fat gadids - Atlantic and Arctic cod (93.5% for both versus 94.7%, 95.7% and 96.6% for halibut, capelin and herring, respectively) (Lawson *et al.* 1997). Similar relationships between digestive efficiencies and energy content of the diet were found within kittiwakes and guillemots fed capelin or cod (Brekke and Gabrielsen 1994). This could potentially have contributed to the decline of marine birds that switched their prey from small forage fish to gadids as well.

All of these studies provide support for the theory that pollock is less nutritious than herring, and that switching from a high quality, diverse diet including herring to one predominantly of pollock could place marine mammals under nutritional stress. What has not been evaluated to date is what exactly renders pollock less nutritious. Whether

the value of pollock is lower than that of herring only because of a difference in energy density, or whether there is something intrinsically missing from pollock or present in pollock that reduces its quality, has yet to be determined.

CHAPTER II: The Effects of Pollock and Herring on Growth and Reproductive Performance in the Laboratory Rat, *Rattus norvegicus*

Introduction

Steller sea lion populations in the Gulf of Alaska and the Aleutian Islands have declined by over 80% since the late 1970's in parallel with an apparent shift in the ocean's composition of fish; most notably an increase in gadids such as walleye pollock and a decrease in Pacific herring (Springer and Byrd 1988; Trites and Larkin 1996; Calkins *et al.* 1999; Schumacher and Alexander 1999; Trites *et al.* 1999). Stomach contents of Steller sea lions reflect this change in availability with a shift in dominant prey species from herring to pollock (Pitcher 1981; Merrick and Calkins 1996). From a physiological context, these dietary changes have been accompanied by reduced body size (length and mass), reduced pup numbers and poor fur quality in Steller sea lions (Calkins *et al.* 1998). One hypothesis linking the above observations is that the sea lions experienced a nutritional deficiency when their dominant prey species shifted from a fatty fish (e.g. herring) to a non-fatty fish (e.g. pollock).

Studies using captive juvenile Steller sea lions have found them unable to maintain body mass on diets of pollock, while sea lions gained weight when fed herring diets (Rosen and Trites 2000b). Studies of mink have also found them also unable to maintain body mass while consuming gadids, even when their intake increased significantly (Leoschke 1961). Unfortunately, captive studies are limited by small sample sizes of Steller sea lions and are unable to examine the subtle effects of diet on

reproduction due to their lengthy reproductive cycle. These shortcomings make it necessary to examine alternative animal models in Steller sea lion research.

The purpose of my study was to examine the nutritional value of pollock and herring food sources using the laboratory rat (*Rattus norvegicus*) – a common model for nutritional studies in mammals, and one that is increasingly being used in studies of marine mammals (e.g. Ross *et al.* 1996; Ross *et al.* 1997).

My study compared the nutritional value of pollock and herring by monitoring diet-induced changes in growth, food intake and utilization efficiencies of rats fed different fish diets. I also examined the effect of pollock and herring diets on reproductive parameters such as gestational weight gain, litter sizes and litter weights.

Methodology

Diet Preparation

Herring and pollock were caught off the coast of Alaska and British Columbia (At Sea Processors and Vancouver Aquarium Marine Science Center) and frozen at -30°C. Within 1 week of arrival, all fish were ground while frozen using a Hobart meat grinder and immediately vacuum packed in 4 mil thick polyethylene bags before being stored at -30°C in opaque boxes to prevent lipid oxidation. The ground fish was freeze-dried (Pleasant Valley Freeze Dry) and mixed into one of four experimental diets (Table 5). Freshly mixed diets were immediately divided into 1 kg bags (approximately 3 days worth of feed), vacuum packed and stored in opaque boxes at -30°C until use. Once opened for use in experimental feeding, the diets were stored in air-tight plastic

Table 4. Proximate composition analyses and energy content of fish used in experimental diets. Reported as mean percentages of 10 independent samples. All values reported on a dry matter basis. GE = gross energy, ME = metabolizable energy.

	Protein	Fat	Fiber	Ash	GE (Mcal/kg)
Pollock	64.57	25.55	1.96	11.39	6.24
Herring	54.28	43.84	0.78	8.70	7.29

containers at 5°C for a maximum of 3 days. Peroxide values of the diets were taken on the first day of use and after two days of refrigeration (AOAC Official Method 965.33, 2000) and did not exceed 2 mequiv/kg fat.

Ten fish of each species were analyzed for protein, fat, ash, fiber and moisture content as well as energy content to ensure that all of the diets fed to my study animals were balanced (Norwest Labs, Vancouver) (Table 4). Diets were formulated using ICN (Aurora, Ohio) semi-purified diet components, the freeze-dried pollock and herring, and pollock and herring oil (At Sea Processors and West Coast Reduction) (Table 5). Diets contained exact proportions of all ingredients of ICN's semi-purified AIN-76 powdered rat diet, less the protein and fat components. Protein (held constant at 20%) and fat were provided through the freeze-dried fish and through added fish oil in two diets. The first diet (P) contained pollock as the protein source and the second diet contained herring (H), with fat values being those naturally occurring in these particular fish. The third diet contained pollock supplemented with pollock oil (PP), and the fourth diet was pollock supplemented with herring oil (PH). These supplemented diets both contained the same concentration of fat as the herring diet, and were therefore isocaloric to the herring diet

Table 5. Compositions, gross energy and protein content of the five experimental diets. Values are percentages unless otherwise stated. Values with different superscripts are significantly different.

	Herring (H)	Pollock + Herring Oil (PH)	Pollock (P)	Pollock + Pollock Oil (PP)	ICN (ICN)
Fish protein	20.0	20.0	20.0	20.0	0.0[1]
D-L methionine	0.3	0.3	0.3	0.3	0.3
Corn starch	15.0	15.0	15.0	15.0	15.0
Sucrose	38.15	35.8	44.0	35.8	50.0
Alflacel	5.0	5.0	5.0	5.0	5.0
Natural oil[2]	16.16	7.9	7.9	7.9	5.0
Mineral mix	3.5	3.5	3.5	3.5	3.5
Vitamin mix	1.0	1.0	1.0	1.0	1.0
Choline bitartrate	0.2	0.2	0.2	0.2	0.2
Added oil	0.0	8.26	0.0	8.26	0.0
Gross Energy (cal/g)	4840.8^{b}	4955.2^{b}	4415.7^{a}	4993.3^{b}	4476.8^{a}
Protein Content	17.13^{c}	18.37^{c}	17.06^{c}	18.76^{c}	18.83^{c}

[1]Protein source in ICN diet is casein (20%).
[2]Oil content of fish in fish diets and corn oil in the ICN diet.

and effectively created "fatty pollock". The difference between these two diets was the source of the fish oil (e.g. from pollock or herring) therefore enabling a comparison of the two fish oils. The fifth diet was a control diet, (ICN), being semi-purified AIN-76 rat diet, and was isocaloric with the pollock diet. This diet was designed to ensure that responses obtained from the pollock group were due to the ingestion of fish, and were not the result of caloric intake (due to the low fat content of pollock versus herring). Energy and protein contents of all diets (Table 5) were confirmed prior to experimental feeding to ensure that 1) diets P and ICN were isocaloric, 2) diets H, PP and PH were isocaloric, and 3) that all contained equal proportions of protein.

Fatty acid profiles for all diets were determined in duplicate by gas chromatography. Lipids were extracted from diet samples with chloroform/methanol (2:1) by the method described by Folch *et al.* (1957), methylated with H_2SO_4, and analyzed for component fatty acids using a Shimadzu 3700 GC according to the method of Nwokolo and Kitts (Nwokolo and Kitts 1988).

Fatty acid standards were ordered from Sigma, Inc. The fatty acid standard mixture included methyl esters of the following fatty acids: C12:0, C14:0, C16:0, C16:1n-9, C17:0, C18:0, C18:1n-9, C18:2n-6, C18:3n-3, C20:4n-6, C20:5n-3, C22:6n-3.

Amino acid profiles were also obtained for all diets (method JAOAC Vol. 71, No. 6, 1988 by SGS Laboratories, Vancouver).

Animal Protocols

A total of 60, weanling (~22 days old) female Wistar rats (University of British Columbia breeding colony) were randomly divided into five groups (12 per group) and assigned to one of the above five diets (Table 5). Rats were acclimatized to the lab and physical handling for 3 days prior to data collection. The animals were housed in stainless steel cages in a room with ambient temperature set at 21 ± 1°C, with 10 air changes per hour and controlled lighting conditions (12:12 light:dark). All procedures were in accordance with the University of British Columbia's Committee on Animal Care and with the guidelines of the Canadian Council of Animal Care. Food and water were made available *ad libitum* until 9:00 am every morning, with the exception of the ICN group which was meal fed with the P group. Fresh food was provided each morning after all measurements were taken. Portion dishes were washed daily, and uneaten food was weighed and discarded immediately.

Growth Parameters

Body weights were recorded three times a week. Length (crown to rump) and girth (measured just anterior of the pelvic girdle) were recorded once a week.

Body condition index (CI) for each rat was calculated as

1] $$\mathbf{CI = M / \hat{M}}$$

where "M" is the measured mass and "^M" is the expected mass from a repeated measures regression of mass on length (Figure 3). Fur quality was recorded weekly (by the same individual) and scored on a scale of 1-5, with one representing dry, discolored fur and five representing rich, white fur.

Food Intake

Each morning, food spillage was retrieved from trays under the cages and added to each rat's leftover food. This was subtracted from the amount of food supplied the night before and recorded as food intake. On the rare occassion that water or urine had wet a rat's spilled food, food intake was not recorded for that day.

Efficiency of Energy Utilization and Protein Efficiency Ratio

Efficiency of energy utilization (EU) was calculated as the weight gain over 9 weeks, divided by the calories consumed (Harris 1991; Mehta *et al.* 1994; Iossa *et al.* 1997)

2] **EU = weight gain (g) / calories consumed (cal)**

Protein efficiency ratio (PER) was calculated as the weight gain over 9 weeks, divided by the amount of protein consumed (Muller and Tobin 1980).

3] **PER = weight gain (g) / protein consumed (g)**

Reproductive Parameters

As the rats approached puberty (~35 days old), they were examined daily for vaginal opening as evidence of first estrus.

Starting at an age of approximately 13 weeks, rats were mated using a staggered, individual, overnight breeding schedule. The first morning on which copulatory plugs were observed or vaginal lavages showed sperm under a compound microscope was termed Day 0 of gestation. On day 19 of gestation (two days prior to parturition), rats were weighed and sacrificed under halothane anesthetic (Bimeda-MTC, Cambridge, ON). Fetal and placental weights, the number of corpora lutea, total conceptus weights (fetuses within uterus), ovarian weights and litter size (number of fetuses per female) were recorded.

Statistics

Differences between groups in all measured parameters were analyzed by analysis of variance or repeated-measures analysis of variance unless otherwise stated. Differences were considered significant at $p \leq 0.05$ unless otherwise stated.

Results

Diet Analyses

The fatty acid profiles of all five diets are shown in Table 6. The ratio of polyunsaturated (PUFA) to saturated fatty acids was highest for the ICN diet (3.30) and lowest for the H diet (0.38). The n-3: n-6 ratio was highest for the P diet (15.10), intermediate for the H diet (7.71), and lowest for the ICN diet (0.01). As expected, fish diets were high in long-chain PUFA, with the P diet containing almost twice as much eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) as the H diet. The ICN diet containing corn oil was high in linoleic acid (C18:2n-6), but did not contain long-chain PUFA. Herring oil was highest in oleic acid (C18:1n-9).

The amino acid profiles for all diets are shown in Table 7, along with the nutritional requirements of the rat (Warner and Breuer 1972). Intra-assay variation between replicate analyses on protein standards by SGS Laboratories was less than 2%. The PH and PP diets contained slight deficiencies in isoleucine, and the PH diet was also slightly low in phenylalanine.

Table 6. Fatty acid compositions (mass basis % of fatty acids), PUFA:saturated and n-3:n-6 ratios of treatment diets and oils used in the formulation of supplemented diets. Values are means of duplicate analyses.

	Pollock Oil	Pollock	Pollock + Pollock Oil	Herring Oil	Pollock + Herring Oil	Herring	ICN
10:0	0.21	0.48	0.28	0.12	0.24	0.23	0.17
14:0	8.02	8.36	8.40	4.06	5.83	6.68	0.32
16:0	22.01	25.34	23.61	25.97	24.29	31.65	12.55
16:1	16.47	11.77	14.59	7.21	9.39	7.18	0.10
18:0	3.78	5.67	4.57	4.90	4.46	4.38	3.09
18:1(n-9)	29.27	21.68	25.51	47.43	37.23	33.68	30.46
18:2(n-6)	1.25	1.28	1.39	0.98	1.10	1.56	52.49
18:3(n-3)	0.46	0.22	0.42	0.29	0.38	0.64	0.82
20:4(n-6)	0.51	0.38	0.45	0.17	0.26	0.30	0.00
20:5(n-3)	12.21	14.79	13.28	5.31	10.01	7.94	0.00
22:6(n-3)	5.81	10.03	7.50	3.56	6.81	5.76	0.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
PUFA:sat	0.56	0.67	0.62	0.29	0.53	0.38	3.30
n-3:n-6	10.50	15.10	11.50	7.96	12.66	7.71	0.01

Table 7. Amino acid profiles (% dry matter) of treatment diets and requirements of the laboratory rat. Values are means of duplicate analyses.

	Herring	Pollock + Herring Oil	Pollock	Pollock + Pollock Oil	ICN	Rat Requirements
Alanine	1.89	1.38	1.50	1.37	0.98	
Arginine	1.23	1.04	0.91	1.05	0.78	0.67
Aspartic Acid	2.10	1.89	1.67	1.96	1.36	0.44
Cystine	0.15	0.28	0.32	0.20	0.50	
Glutamic Acid	3.10	2.80	3.13	2.67	4.76	4.4
Glycine	1.87	1.70	1.57	1.28	0.93	
Histidine [1]	0.51	0.44	0.42	0.59	0.50	0.33
Isoleucine [1]	1.14	0.51[4]	0.93	0.58[4]	0.83	0.61
Leucine [1]	1.77	1.39	1.55	1.43	1.69	0.83
Lysine [1]	1.67	1.59	1.74	1.57	1.42	1
Methionine [1]	0.49	0.55	0.67	0.48	1.01	0.67[2]
Phenylalanine [1]	0.70	0.52[4]	0.75	0.67	0.74	0.89[3]
Proline	1.70	0.87	1.04	0.96	3.81	0.44
Serine	1.26	1.20	1.10	1.07	1.40	
Threonine [1]	1.23	1.24	0.85	0.93	0.93	0.56
Tryptophan [1]	0.15	0.17	0.19	0.16	0.56	0.17
Tyrosine	0.35	0.32	0.30	0.31	0.69	
Valine [1]	1.74	0.93	1.50	1.28	1.52	0.67

[1] Essential amino acid
[2] One third to one half may be supplied by L-cystine
[3] Phenylalanine-tyrosine; one third to one half may be supplied by L-tyrosine.
[4] Less than requirements according to Warner and Breuer (1972)

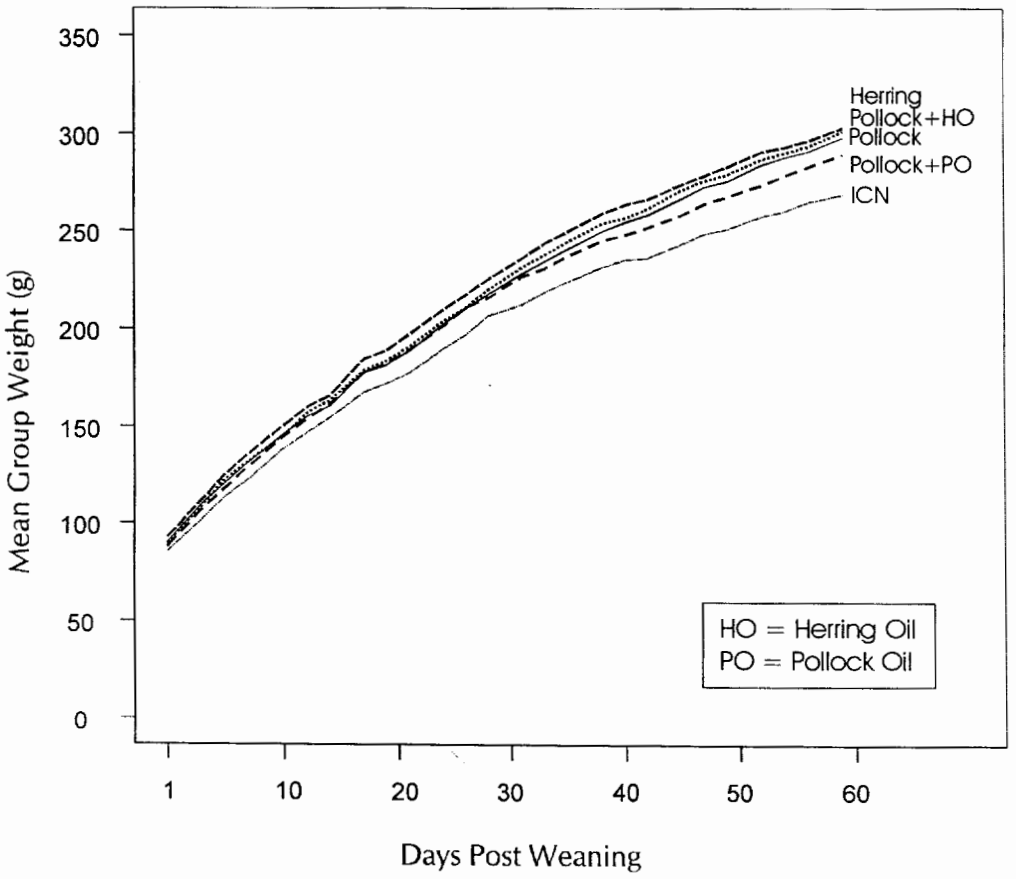

Figure 1. Mean group body weights for rats fed different fish diets or a semi-purified ICN diet (n=12 per diet). ICN rats weighed significantly less than Herring, Pollock + HO and Pollock rats on Day 60.

Growth

The growth curves for each diet group prior to mating are shown in Figure 1. On day 60, rats fed the H, PH and P diets were significantly larger than rats eating ICN ($F_{[4,55]} = 3.519$, p=0.002). The total weight gains from weaning to sacrifice (day 19 of pregnancy) are shown in Figure 2. ICN rats were significantly smaller than all rats fed fish diets ($F_{[4,44]} = 6.147$, p=0.0005). A trend for H rats being heavier than all pollock groups (P, PP and PH) was obtained, although this difference was not significant.

Figure 3 shows the significant correlation between mass and length of rats prior to mating, and the repeated-measures linear regression (r^2=0.9211, n=60, p=0.001) used to calculate the body condition index (CI). Rats fed the P diet had a significantly higher condition index than rats fed ICN diet throughout the first 8 weeks ($F_{[4,55]}$=2.56, p=0.0084), but there were no differences in condition between groups eating fish diets (Figure 4). There were no differences in quality and condition of fur between all groups.

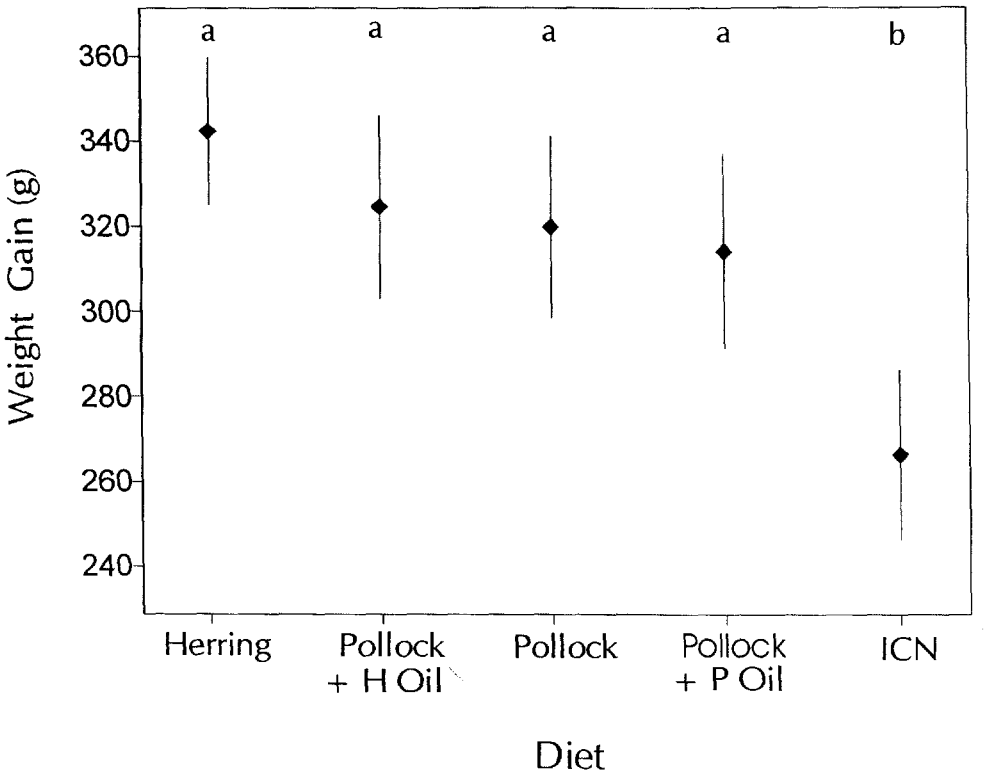

Figure 2. Mean total weight gain from weaning to Day 19 of pregnancy. Values are mean weight gains (minus weight of conceptus) and 95% confidence intervals. Rats were randomly mated; age at Day 19 of pregnancy ranged from 13-18 weeks. Values with different subscripts are significantly different.

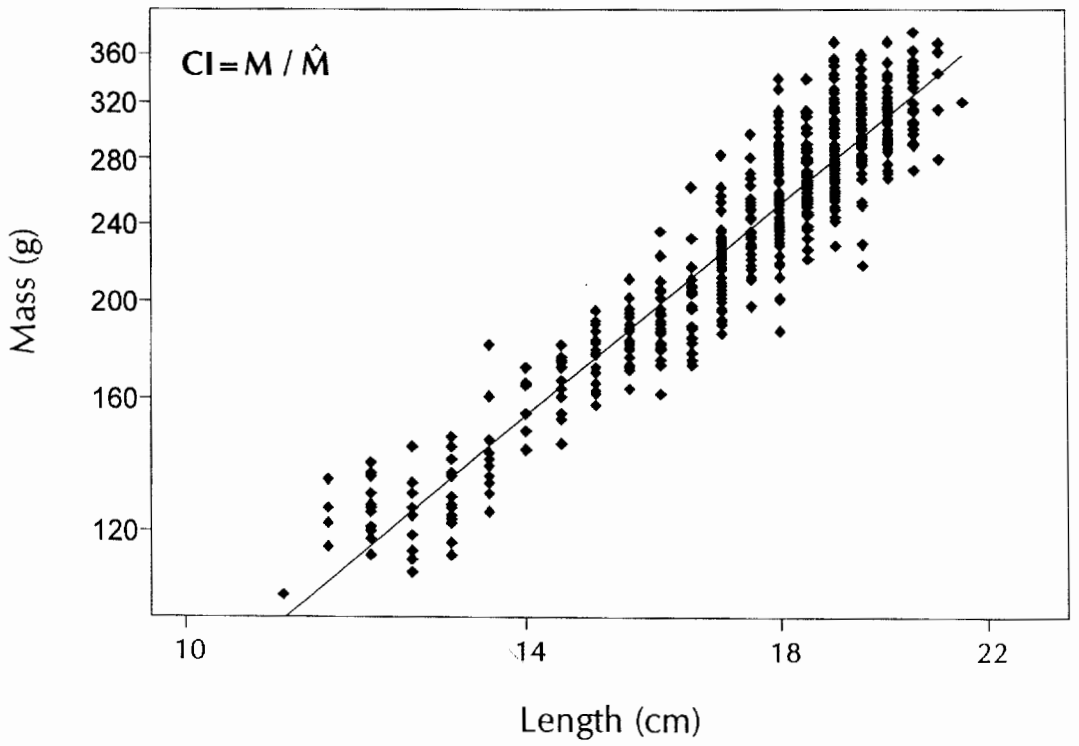

Figure 3. The relationship between length and mass in Wistar rats used to calculate the body condition index (CI). CI = mass / expected mass. Repeated-measures regression (ln-ln): $y = 2.1538x - 0.7836$, $n = 60$, $r^2 = 0.9211$, $p<0.001$.

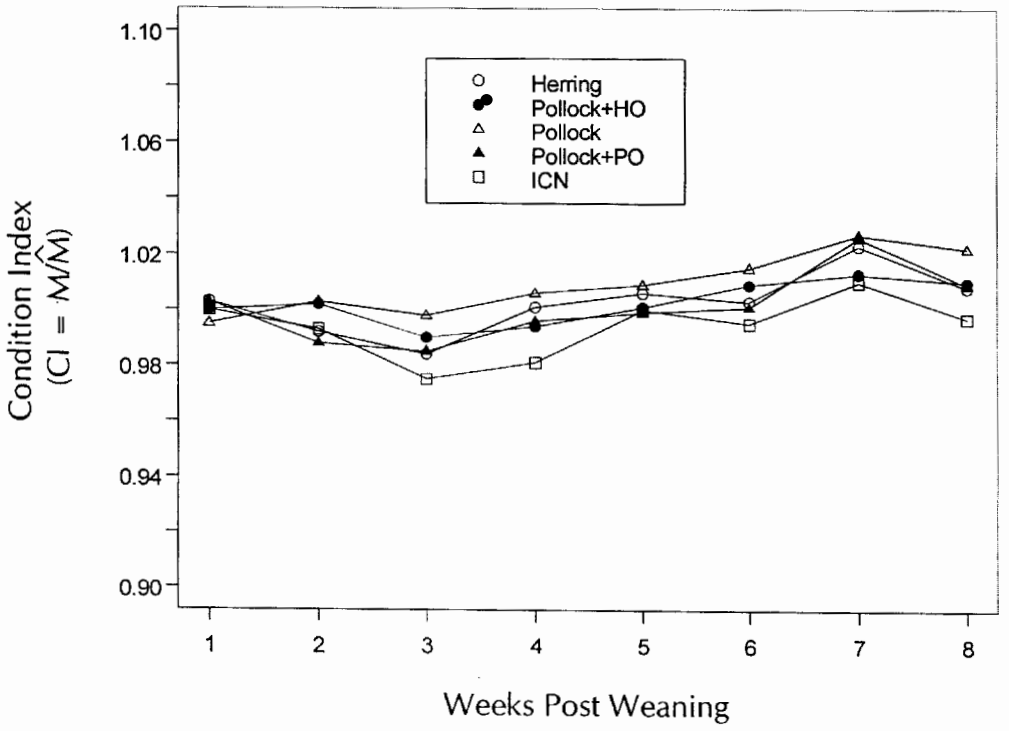

Figure 4. Condition indicies of rats fed different fish diets or ICN diet from weaning to 8 weeks post-weaning (n = 12 per group).

Food Intake

Mean food intakes and caloric intakes prior to mating are shown in Figure 5. Food intakes for rats fed the lower calorie diets (P and ICN) were significantly higher than those fed the higher calorie diets (H, PH and PP) ($F_{[4,55]}$=17.025, p<0.001). The difference in energetic density of the diets was ~10% (Table 5), and intake was ~10% higher in the groups consuming the lower calorie diets. Caloric intake, therefore, was equal in all groups ($F_{[4,55]}$=2.205, p=0.08).

Efficiency of Energy Utilization and Protein Efficiency Ratio

Energy utilization was highest for rats fed P (4.31×10^{-5} g/cal), and significantly reduced for rats fed ICN (3.93×10^{-5} g/cal) ($F_{[4,55]}$=2.968, p=0.027) (Figure 6). Adding both herring and pollock oil to the pollock diets slightly reduced this efficiency of energy utilization, although not significantly.

Protein efficiency ratio was highest for rats that were fed herring (1.19) and lowest for ICN rats (0.93) ($F_{[4,55]}$=18.702, p<0.001). Rats fed pollock, regardless of fat content, had protein efficiency values between 1.09 and 1.12.

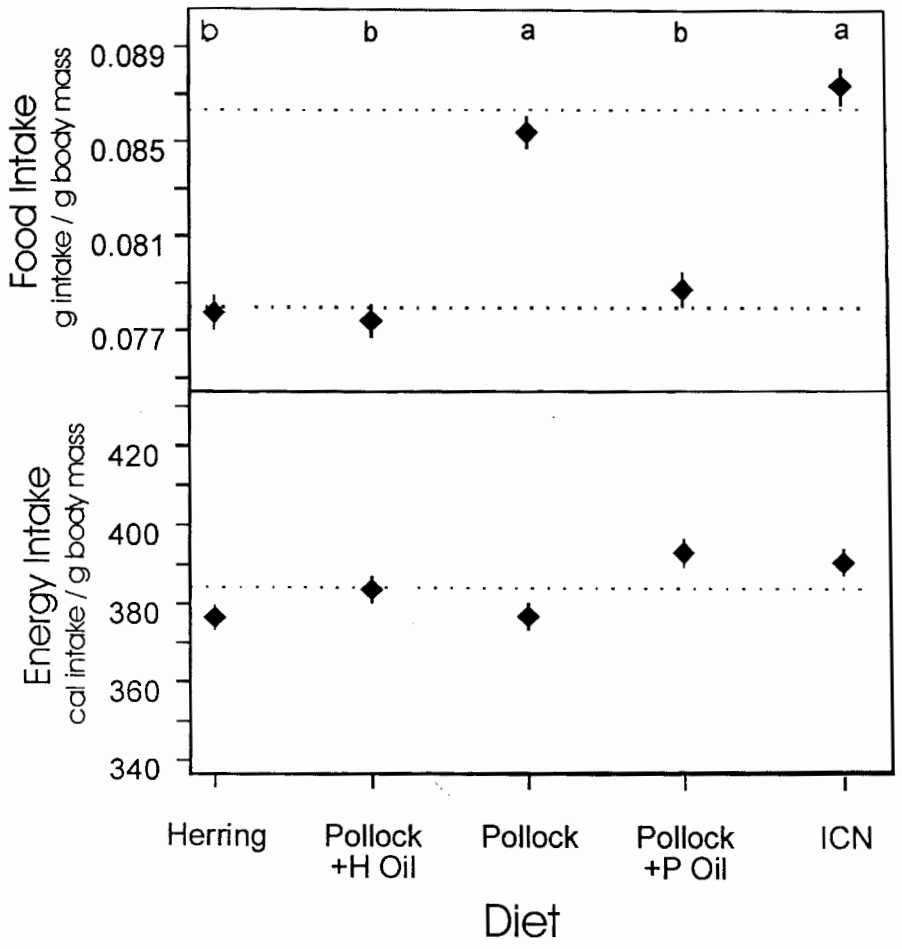

Figure 5. Mean daily food and caloric intake of non-pregnant rats fed different fish diets or ICN diet. Values are means and 95% confidence intervals (n = 12 per group). Values with different subscripts are significantly different. Dotted lines are means of statistically similar groups.

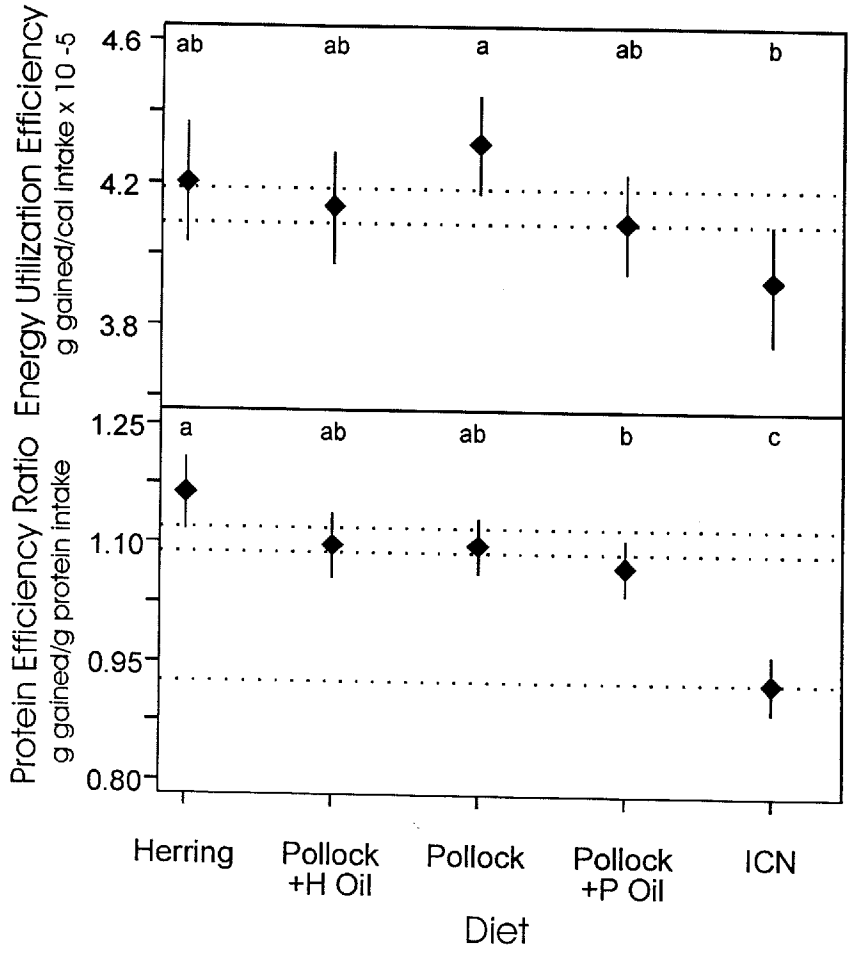

Figure 6. Mean efficiencies of energy utilization and protein efficiency ratios of rats fed different fish diets or ICN diet. Values are means and 95% confidence intervals (n = 12 per group). Values with different subscripts are significantly different. Dotted lines are means of statistically similar groups.

Reproduction

Mass corrected daily food intake during pregnancy ranged from 0.061 g/g body mass to 0.066 g/g body mass. There were no significant differences in food intake among dietary treatments (Table 8). Mass corrected daily caloric intake ranged from 285.81 cal/g body mass to 307.69 cal/g body mass. Again, there were no significant differences in energy intakes observed. The mean energy intake for all rats during pregnancy was 105 kcal/day.

Mean litter size (13.5 fetuses) and gestational weight gain (53.1g) did not differ significantly between all dietary groups (Figure 7). Nor was there a difference in the interval from birth to first estrus between groups, the mean age being 43 days.

There were no differences in the number of corpora lutea, nor in total conceptus, ovarian or placental weights on day 19 of pregnancy (Table 8). Mean fetus weights of rats fed P were significantly smaller (1.96 g) than rats fed H, PP and ICN (2.11, 2.10 and 2.14 g, respectively) ($F_{[4,45]}$=8.47, p<0.001).

Table 8. Reproductive and tissue parameters of rats fed different fish diets or a semi-purified ICN diet. Measurements taken at sacrifice on Day 19 of pregnancy. Values are means and standard errors. Values with different superscripts are significantly different.

	Herring N=11	Pollock + Herring Oil n=11	Pollock n=11	Pollock + Pollock Oil n=9	ICN n=9
Food Intake[1] (g/g body mass)	0.063 (.002)	0.061 (.002)	0.065(.001)	0.062(.002)	0.066(.003)
Caloric Intake[1] (cal/day)	306.22 (10.94)	302.80 (10.76)	285.81 (7.1)	307.69 (9.26)	294.75 (13.5)
Mean Fetus weight (g)	2.11^{b} (.03)	2.02^{ab} (.02)	1.96^{a} (.03)	2.10^{b} (.02)	2.14^{b} (.02)
Conceptus Weight (g)[2]	54.12 (3.53)	51.00 (4.11)	51.92 (3.06)	47.74 (5.45)	52.51 (3.88)
# Corpora lutea	16.63 (.74)	17.09 (.44)	15.70 (.75)	15.56 (.75)	14.78 (1.15)
Ovarian Weight (g)[3]	0.20 (.01)	0.23 (.02)	0.22 (.02)	0.21 (.06)	0.20 (.02)
Placental Weight (g)[4]	5.40 (.30)	5.01 (.39)	5.35 (.33)	4.74 (.52)	5.04 (.33)

[1] Mass corrected mean daily intake or caloric intake per day from Day 1 to Day 19 of gestation.
[2] Weight of all fetuses, uterus, ovaries and uterine fluids.
[3] Mean of left and right ovary.
[4] Mean weight of all placentas per female.

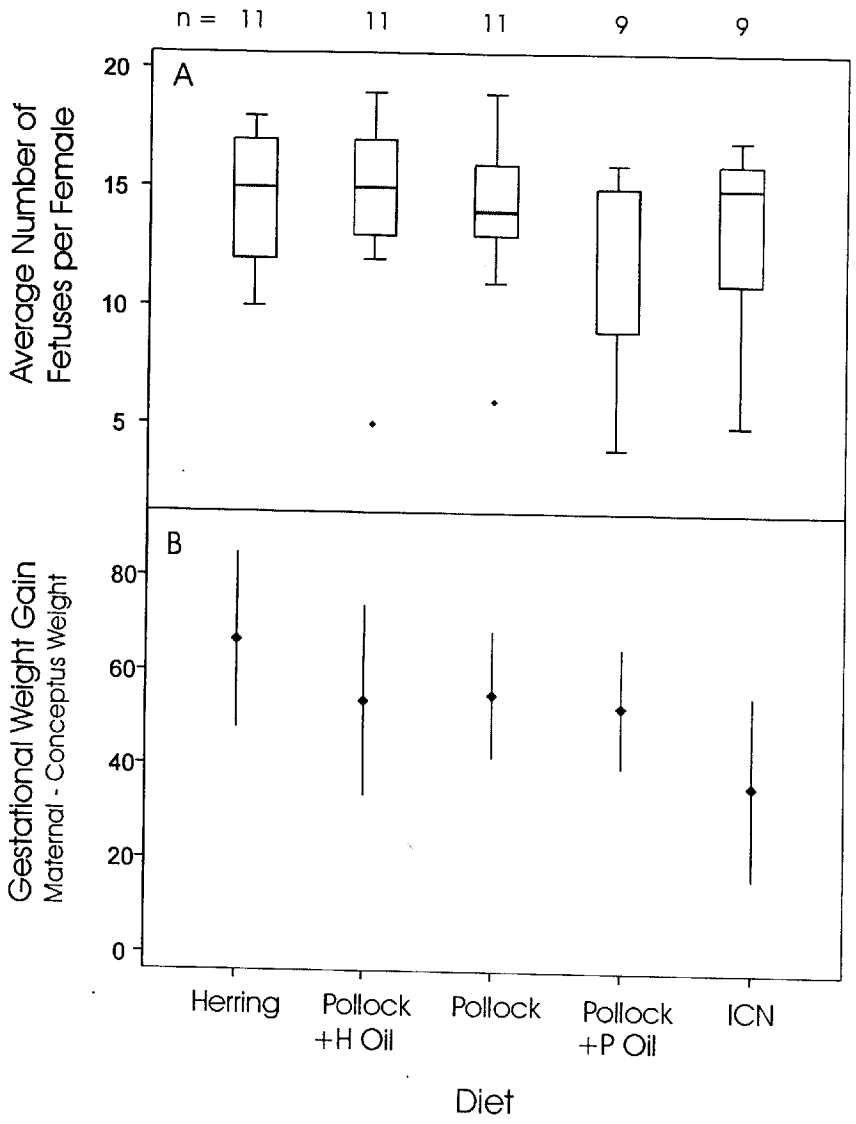

Figure 7. Litter size and maternal weight gain of female rats fed different fish diets or ICN diet. A. Medians are represented by a horizontal line within each box plot. B. Gestational weight gain is the maternal weight gain during pregnancy minus the weight of the uterus, fetuses and all fluids. Values are means and 95% confidence intervals.

Discussion

Growth

My study was conducted to determine whether the physiological changes observed in the declining population of Steller sea lions could be caused by the consumption of walleye pollock (a dominant prey in Alaska). One of those changes in sea lions was reduced body size (weight and volume) as compared to pre-decline measurements when pollock consumption was lower (Calkins *et al.* 1998). In my study, I found that all groups of rats fed fish diets (regardless of oil content or source) had statistically similar growth prior to reproduction. However, mean growth curves showed a uniform trend, with rats fed herring (H) being consistently heavier than those fed pollock supplemented with herring oil (PH), who were in turn heavier than those fed pollock (P) and pollock supplemented with its own oil (PP) (Figures 1 and 2). Power analysis indicates that such differences would be statistically significant if I increased my sample size from 12 to 22 rats per treatment group. Whether the differences I observed were due to the quality of fat, the quality of the fish proteins, or the amount of fat present in the diets is debatable.

Quality of Fat

Growth curves similar to those I obtained have resulted from feeding rats fat-free basal diets supplemented with 10% menhaden, tuna or herring oil (Privett *et al.* 1960), thereby supporting the notion that fish oils from a range of marine species are a complete source of essential fatty acids (Stansby 1969). Thus, differences in the growth of my rats were not entirely attributed to fat quality, as defined by the fatty acid composition of the

fish oils. By the end of the study (Figure 2), the type of oil added to the pollock diet (herring oil or pollock oil) seemed to have no effect on final weight. It may be concluded, therefore, that not only were the fatty acid compositions of the oils complete, but digestibility of the lipid source was also not a limiting factor.

Corn oil, which served as a control source of fat in my study and others (Privett *et al.* 1960; Zhang *et al.* 1992; Mehta *et al.* 1994), consistently results in growth below that of groups fed fish oil, and is discussed later with reference to its fatty acid composition (see *Diet Quality* section).

Quality of Protein

Differences in the quality of dietary protein were not obvious from the pre-pregnancy growth curves. Although all groups receiving pollock protein seemed to be lighter than those receiving herring, the differences were not significant at this sample size. Herring protein has been shown to be superior in its ability to promote growth as compared to 17 other species of fish protein, including salmon, tuna and halibut (Nilson *et al.* 1947). Differences in protein quality in this study became more pronounced after reproduction (Figure 2). By this time, the rats that received herring seemed to be heavier than those that received pollock, regardless of the type of oil or the amount of oil consumed. The superior quality of herring protein is supported by its elevated protein efficiency ratio as compared to all pollock diets (Figure 6).

Quantity of Fat

The quantity of fat in the diets is a third possible factor that could cause differential growth between groups. The P and the ICN diets contained 8.25% less fat than the herring and supplemented pollock diets (PP and PH). However, the growth of the rats fed pollock (P) was higher than the ICN group. This suggests that the fat content of pollock did not limit growth. Higher fat contents meant higher energy contents, and should have promoted higher weight gains in rats consuming the fattier diets. The reason I did not see this is because the rats compensated for the lower energy density of the diets by increasing their food intakes.

Food Intake

Rats ate more of the low calorie diets (P and ICN) compared to the other three diets. This resulted in equal energy intakes among all groups (Figure 5). This compensation response is consistent with other studies that have shown that rats can regulate the type and amount of food items they consume (Johnson *et al.* 1986; Harris 1991; Ackroff and Sclafani 1996; Shaw *et al.* 1997; Hempenius *et al.* 2000). Rats fed energetically dilute feeds can increase their food intake by as much as 60% (Johnson *et al.* 1986).

The mechanisms that control caloric intake in rats are poorly understood. There is evidence both supporting and rejecting the notion that many mammals regulate food intake based on fat content. For example, one study compared the intake of a high fat diet versus a low fat diet, and found that rats regulated caloric intake rather than fat intake (Harris 1991). Conversely, another study found that the presence of fat in the upper

small intestine reduced food intake and prolonged postprandial satiety in rats, most likely through secretion of cholecystokinin and serotonin (Burton-Freeman *et al.* 1999). It has also been shown that the magnitude of food intake inhibition is a function of the concentration of lipid used in the feed, and that the site of this satiation is in the duodenum in Sprague-Dawley rats (Greenberg and Smith 1997). Thus, this particular satiating effect is preabsorptive. The chemical structure of the fat or oil also affects the level of satiation, with longer and more unsaturated fatty acids eliciting greater satiety effects (Greenberg *et al.* 1988).

An alternative and widely accepted "depletion-repletion" model of food intake states that feeding is initiated when blood or brain levels of metabolites, termed appetostats, fall below a certain threshold level (Johnson *et al.* 1986). Such appetostats may be circulating glucose and gastrointestinal peptides (see review by Schwartz *et al.* 1999). Glucose levels appear to drop immediately prior to meal initiation in rats. However, it is unclear whether this is the cause of meal initiation or an anticipatory response to nutrient ingestion. The gastrointestinal peptide, cholecystokinin, causes rats to consume less when injected intraperitoneally, and possibly promotes satiety and inhibits food intake by inhibiting gastric emptying and therefore prolonging stomach distension. The importance of stomach distension in food intake is supported by studies that found that rats increased meal frequency of low calorie diets rather than increasing meal size, and by other studies that found rats had a tendency to overeat liquid diets as compared to those eating bulkier, solid food diets (Johnson *et al.* 1986; Ackroff and Sclafani 1996).

Evidence contrary to the depletion-repletion model suggests that caloric intake is regulated over a span of several meals or days, rather than meal to meal, indicating that food intake may be controlled by more than immediate levels of metabolites that control food intake (Johnson *et al.* 1986; Beck *et al.* 1990). The recently noted importance of leptin, insulin and neuropeptide Y (NPY) in energy balance and food intake represent long-term controls of energy balance and adiposity, and can in fact override shorter-term satiety cues such as glucose and gastric peptides to maintain energy balance (Schwartz *et al.* 1999).

My finding that rats increased their intake of pollock to compensate for its low energetic density is important. To date, there is no evidence that Steller sea lions can or will increase their intake of pollock to compensate for a less energetically dense food source. Indeed, one study found that they may lose body mass on *ad libitum* pollock diets (~0.6 kg/day) (Rosen and Trites 2000b). Juvenile harp seals switched from a diet of high calorie Atlantic herring to low calorie Atlantic pollock did not increase the amount of food they consumed to make up for the caloric density of the two prey species. Instead of losing mass, however, they lost 32% of their fat reserves and gained lean body mass (Kirsch *et al.* 2000). Young mink fed low fat gadids such as cod, haddock, whiting and hake, have been shown to increase their food intake to compensate for low energy (Leoschke 1961). However, their growth was still retarded compared to those fed higher fat fish (the optimal fat content being approximately 25% for this aquatic mammal, Leoschke 1961). The ability of rats to compensate for low energy pollock diets and maintain satisfactory growth suggests that there is either a palatability issue, or that there are innate differences in food intake and satiation regulation in sea lions and other

mammals. It is also possible that the feeding studies with captive Steller sea lions have not been long enough for palatability issues (if this is indeed a factor) to be overridden by energetic demands.

If increased intake of pollock is physiologically possible for sea lions, there would be an enormous energetic cost to this increase. Estimates derived from the energetic compositions of herring and pollock, the heat increments of feeding of both diets, digestive efficiencies and urinary energy losses have suggested that sea lions would require 35-80% more pollock than herring to maintain equal net energy intakes (Rosen and Trites 2000b). However, such an increase may not be feasible for an animal that travels up to 350 km per foraging trip (Merrick *et al.* 1995). Proximate analyses of the fish used in my study indicates that a sea lion would require 60% more pollock than herring, which falls well within the range outlined by Rosen and Trites (2000b; assuming the same digestive efficiencies, urinary losses and HIF). The compensatory intake of only 10% in my study was due to the dilution of fish in the diets when all other ICN components were added. This resulted in a difference in energetic density of only 10% between pollock and herring.

Diet Quality

Fatty Acid Content of Diets

The fatty acid profiles of the diets used in my study (Table 6) are similar to profiles in the literature for both herring and corn oil (Stansby 1969; Stansby 1986; Mehta *et al.* 1994). A profile for pollock oil was not found. It must be mentioned that as

is the case with all fish species, fatty acid content can vary substantially across individuals, time, geographic area and season as the diet of the fish changes (Stansby 1986). Certain fatty acids have been shown to fluctuate by more than 14% across these parameters (Stansby 1986). I did not attempt to describe a "typical" fatty acid profile for pollock or herring with these values. However, my fatty acid profiles should be sufficient given that my study attempted only to evaluate the diet fed to my rats and assess the presence or absence of essential fatty acids.

According to the National Academy of Sciences, the fatty acid considered essential for rats is linoleic acid (18:2 n-6), required at a level of 0.24% of energy in the diet (assuming 4 kcal/g diet) (Warner and Breuer 1972). The diet containing the lowest quantity of linoleic acid in my study was PH, and this was still above the minimum requirement, containing 0.3% linoleic acid. Symptoms of essential fatty acid deficiency include reduced growth, scaly skin, rough and thin hair, irregular estrus, prolonged gestation, frequent fetal resorptions and litters of low viability (Warner and Breuer 1972). The absence of any of these symptoms supports the notion that all fish diets supplied adequate quantities of essential fatty acids.

Despite linoleic acid quantities in the ICN diet being almost 50 times higher than the fish diets, the ICN group exhibited reduced growth and slightly smaller litter sizes, possible symptoms of fatty acid deficiencies. The ICN diet contained solely corn oil, void of long-chain fatty acids such as arachidonic acid (C20:4 n-6), which is considered by some to be an essential fatty acid due to the low efficiency of conversion from linoleic acid (Arrington 1978).

The n-3 (linolenic) and n-6 (linoleic) fatty acids are metabolized through competing pathways involving alternating desaturations and elongations in the formation of long-chain polyunsaturated fatty acids (PUFA) (Holman 1997). However, the n-3 acids are more persistently conserved and may be optimally consumed at a ratio of 1:4 (n-3:n-6) because of the uneven relationship. How much of a deviation from this ratio is acceptable is not known.

The fatty acid profiles of my diets suggest that the fish fed to the rats were relatively high in n-3 fatty acids and that the ICN diet was extremely high in n-6 fatty acids. Elevated dietary n-3 fatty acid content, such as that in the fish diets, may have less adverse effects than excessive n-6 fatty acids. This is because reduced n-3 fatty acids results in excessive n-6 metabolism, which can have disadvantageous effects on heart health and immune responses (Wainwright 1997). A balance between the n-3 and n-6 fatty acids must be maintained for normal metabolism. A lack of balance, particularly with n-3 deficiency, can lead to adverse health effects such as heart disease, impaired nerve function and altered vision (Carlson 1991).

Corn oil does not contain two other important long-chain PUFA, namely eicosapentaenoic aced (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3). The precursor to EPA and DHA is linolenic acid (18:3n-3), which is most adundant in the ICN diet (Arts *et al.* 2001). However, as with the conversion of linoleic acid to arachadonic acid, conversion of linolenic acid to DHA and EPA is highly restricted and too inefficient to provide animals with adequate amounts of these long-chain PUFA (Arts *et al.* 2001). Additionally, high levels of n-6 fatty acids, such as linoleic acid in the ICN diet, can further reduce this conversion by 40-50 %, making deficiencies in these PUFA

very possible (Arts *et al.* 2001). DHA and EPA are crucial to brain development, normal retinal function and the regulation of eicosanoid formation. Eicosanoids are necessary for immune responses and normal platelet function, but can be detrimental at high levels (Dratz and Deese 1986; Lands 1986; Weber *et al.* 1986). Linoleic acid, comprising 50% of the fatty acids in corn oil, is the preferred precursor of arachidonic acid, which is itself a precursor to the eicosanoids (Dratz and Deese 1986). Eicosanoid production can be inhibited by the n-3 fatty acid, linolenic acid, or EPA and DHA. In the absence of these fatty acids, namely the n-3 fatty acids that are not present in corn oil, eicosanoid levels may be too high and may have negatively effected my rats (Dratz and Deese 1986; Weber *et al.* 1986).

Human patients fed diets rich in n-6 fatty acids and devoid of n-3 fatty acids experienced symptoms of fatty acid deficiency such as numbness, weakness, leg pain, psychological disturbances and blurred vision, all of which would not be easily detected in my rats. In fact, fatty acid deficiency can be difficult to prognose in rats even after prolonged exposure to fat deficient diets (Lands *et al.* 1991). The n-3 fatty acids, particularly DHA and EPA, are also of great importance for development of fetuses and neonates, whose rapidly growing brains are over 50% n-3 fatty acids (Holman 1997). The apparent lack of n-3 fatty acids in corn oil could have negatively impacted development of my ICN rats, albeit this was not confirmed in my study.

The pollock diet (P) in my study contained almost twice as much DHA and EPA as the herring diet (H) (Table 6). Thus, the P diet seems superior based on the content of long-chain PUFA. These fatty acids have been correlated with reduced mortality from heart disease in humans and have benefited patients with arthritis and other inflammatory

diseases (Arts *et al.* 2001). However, these benefits may not be valid for a piscivorous animal that probably does not suffer from heart disease and other such aliments.

Given the high variability of fatty acid contents between individual fish and variation over time and space, the significance of the differences between pollock and herring are not known. Whether development of a fetus is consistent once a threshold level of certain fatty acids is attained, or whether there is a dose-related response to some of these long-chain PUFA is also not clear. As of yet, there are no defined "healthy" upper and lower limits established for many components of the diet. The fact that the pollock diet strays farther from the recommended 1:4 ratio of n-3:n-6 fatty acids (15.1 versus 7.71 for herring) may indicate an imbalance in these fish, but the ratio for herring is far from 0.25 as well. On the other hand, pollock seems to be richer in DHA and EPA, two important long-chain PUFA. Unfortunately, this does not explain the reduced growth and litter sizes of the pollock + pollock oil (PP) rats, and the reduced fetal weights of the pollock (P) rats. A comparison of the digestibility and retention of the different oils may prove useful in answering these questions in the future, as well as tissue analyses of the fetuses.

Amino Acid Content of Diets

The essential amino acid content of all diets was relatively complete, and in agreement with literature values (Jacquot 1961; ICN Biomedicals Technical Information, Ohio). Slightly lower levels of phenylalanine and isoleucine in the supplemented pollock diets (as compared to the dietary requirements of rats) are not likely biologically relevant. The published amino acid requirements for rats can vary by approximately 25%

depending on the reference used, and these are generally considered conservative levels (Mitchell 1955; Warner and Breuer 1972). Further, it must be considered that the amino acid proportions are diluted in these mixed diets, whereas a diet wholly consisting of fish would have much higher amino acid contents, and deficiencies for animals eating fish diets are therefore unlikely.

Efficiency of Energy Utilization

The efficiency of energy use by rats consuming the pollock (P) and supplemented pollock diets (PH and PP) was consistent with other studies that have compared high fat and low fat diets. Supplementing a diet with fat reduces the efficiency of energy utilization (Harris 1991; Iossa *et al.* 1997). The addition of pollock/herring oil to the pollock diet could have decreased the viscosity of the meal. Passage rate through the gut has been shown to increase with decreasing viscosity, and can affect the absorption of essential nutrients such as amino acids in growing rats (Larsen *et al.* 1994). The addition of oil to these two diets, therefore, may have increased passage rate and allowed less time for energy absorption in the gut. This may not have been seen in the herring group as the fat in this diet was present in the freeze dried herring, and not added in a liquid state.

Energy utilization efficiencies have been shown to be greater for AIN-76 diet supplemented with fish oil than for AIN-76 supplemented with corn oil or without added oil (Mehta *et al.* 1994), as was demonstrated in my study. This finding supports the idea that fish oils are very high quality oils for growth in mammals.

Other measures of efficiency have been tested in Steller sea lions eating various fish diets. Digestive efficiency, measured as the percentage of energy absorbed from a

diet, has been shown to be highest for herring when compared to pollock, salmon and squid (Rosen and Trites 2000a). Digestive efficiency was positively correlated with energy density; the more fat in the diet, the better the digestive efficiency. My results seem contradictory to this, with herring and the supplemented pollock diets having slightly lower efficiencies than pollock. However, digestive efficiency measures absorbed energy through analysis of food samples and feces, while the efficiency of energy utilization is a less precise measure relating energy intake to mass. If factors such as protein content or quality of protein are more critical to growth than absolute energy intake, then the efficiency of energy utilization would not be the best measure of diet quality.

Protein Efficiency Ratio

The high protein efficiency ratio for herring supports the results of Nilson *et al.* (1947) who found that protein efficiency for herring was the highest (2.31) among 17 species of fish (mean=2.05). Gadids such as pollock were not included in this study, but it does seem that herring protein is of particularly high quality.

Casein has been shown to be an inferior source of protein when compared to fish (Geiger and Borgstrom 1961; Kik 1961), and the superior growth of my rats eating fish, in addition to the significantly lower protein efficiency ratio of the ICN diet, support this finding.

A comparison of Figures 2 and 6 shows a similar trend between total weight gain and protein efficiency ratio. This suggests that protein quality, or the efficiency with which an animal can assimilate its' protein, is a major limiting factor in the growth of

weanling animals and pregnant females. It has been shown that the effects of protein deficiencies, including net protein ratios and utilizations, are much more pronounced in young rats than in adult rats, and especially during pregnancy and lactation when protein requirements are increased (Widdowson 1981; de Mello and Cury 1989; Krajcivicova-Kudlackova and Dibak 1991). Despite the fact that energy consumption was equal in all groups, by the end of my study, their growth seems to have been limited by the quality of the protein.

Reproduction

Age at First Estrus

Puberty marks the onset of reproductive capabilities in young mammals. Nutritional deficiencies have been shown to delay the onset of puberty through reduced circulating leptin, the hormone peptide of the *ob/ob* gene. Leptin, in turn, alters levels of circulating gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH), which control pubertal onset (Bronson 1988; Carlsson *et al.* 1997; Chehab *et al.* 1997; Cheung *et al.* 1997). The initiation of puberty, as determined by vaginal opening, occurs at approximately 42 days of age in Wistar rats (Bennet and Vichery 1970). There was no significant difference in the age at vaginal opening among treatment groups, the mean being approximately 43 days. This implies that treatment groups were not under significant energy restriction, which is supported by the food intake results. Once again, the difference between the diet groups seems to be related to the quality of food, rather than an energy restriction, which would have delayed reproductive function.

Vaginal opening does not necessarily coincide with the first estrus period, with possible lags between vaginal opening and the first ovulation being as long as 7 days (Zhang *et al.* 1992). A difference between dietary groups may have been overlooked because daily vaginal smears were not taken. Additionally, when the rats were delivered to my facility, their age was reported as 24 ± 2 days, which adds significant error to the age at vaginal opening that I observed.

Gestational Food Intake

Energetic requirements during pregnancy increase significantly above maintenance or growth requirements, particularly during the period of most rapid fetal growth near parturition (Widdowson 1981). In humans and pigs, energy requirements during pregnancy increase by 40 % and 175 %, respectively (Curtis 1995; Reese *et al.* 1995). The National Research Council's recommended energy intake for pregnant rats is approximately 76 kcal/day – a 12 kcal/day increase above normal growth requirements (Warner and Breuer 1972). All rats in my study ate beyond this requirement (mean=105 kcal/day) and there was no significant difference in the mass corrected intakes, nor the energy intakes between groups.

The greatest energy requirements for female mammals occur during lactation (Warner and Breuer 1972; Widdowson 1981). For the laboratory rat, required food intake can increase by nearly four times the amount eaten at parturition (Widdowson 1981). Nutritional stress during lactation can result in reduced milk yield and is especially critical under protein deficiencies (Widdowson 1981; McGuire *et al.* 1995). Any form of environmental constraint on food acquisition, therefore, would have greater

impacts on mothers and pups during lactation than during gestation (Veloso and Bozinovic 2000). This is relevant to both my study and to Steller sea lions in the wild. Ideally, my experiment should have continued through lactation to assess the ability of mothers to feed their pups. Pup mortality during the first days of life is another common indicator of reproductive success and would have been a useful parameter in my study (Shaw *et al.* 1997). In mammals such as seals that undergo fasts after parturition, survival of the pups to weaning is closely correlated with the mother's nutritional status. Thus nutrition directly determines reproductive success (Follett 1982).

Gestational Weight Gain & Fetal Weights

The mean gestational weight gain for all rats was 53.1 g, similar to gains attained in studies using similarly aged Wistar rats (mean = 52.9 g) (Yonekubo *et al.* 1993). The differences in gestational weight gain between diets were not significant, but there was a trend towards lower gain in all pollock-eating groups (P, PP and PH), and especially for the ICN group (Figure 7). This trend in weight gain was similar to that of the protein efficiency ratios, again implying that protein quality was a crucial limiting factor during pregnancy. Additionally, lactation may have been more taxing on these groups that gained slightly less weight.

The increasing demands of pregnancy and lactation on a mother are most often met with increasing food intake. But when resources are limited, mothers may use a number of strategies to reduce the impact of a food shortage (Veloso and Bozinovic 2000). These include altering the length of gestation, the duration of lactation, the size of the litter and/or the body mass of her fetus(es).

There is some debate over whether small mothers tend to have multiple offspring with lower birth weights (de Mello and Cury 1989; Abel 1990; Olsen *et al.* 1990; Sohlstrom *et al.* 1994), or whether they have reduced litter sizes (Rowlands and Weir 1984; McGuire *et al.* 1995). The smallest (in weight) group in my study was the ICN group, which had slightly fewer, yet larger, pups. However, the groups eating pollock (PH, P and PP), which were all equal in size and gained similar amounts of weight during pregnancy, did not show this trend. The PP group had slightly reduced litter sizes, and the P group had significantly reduced pup weights. My data do not exclusively support either hypothesis, although I feel that the differences in reproductive parameters are more likely attributable to diet differences than to maternal weight alone.

Nutrition is the principal environmental influence that determines the growth rate of the fetus prior to parturition (Widdowson 1981). The fetuses of rats eating pollock (P) were significantly reduced as compared to other diets. Interestingly, the fetuses of those fed ICN diet did not incur reduced growth. I had expected the birth weights of both groups to be low given that intake compensation was less strict during pregnancy and resulted in slightly reduced energy intakes in the pollock and ICN groups. The ICN rats were able to support their fetuses despite small body sizes and low intake. This might have been due to slightly reduced litter sizes of the ICN rats (i.e., the fewer fetuses a mother has to support, the more energy is available for each of them) (Widdowson 1981). The reasons why the fetuses of rats fed pollock (P) weighed less are unknown, but the inferior quality of pollock protein as seen from reduced protein efficiency ratios could have contributed to this.

There is evidence that even if food intake is adequate during pregnancy, undernutrition early in life (*in utero* and during weaning) can result in smaller litter sizes, litter weights and reduced milk yield during lactation later in life (McGuire *et al.* 1995). Population growth could thus be affected for generations after a population wide food shortage. Additionally, although low birth weight pups may be able to make up mass during suckling and adolescence if food supply is adequate, deficiencies early in life may result in permanently smaller skeletal structures (Ohlsson and Smith 2001). This can have drastic repercussions for males that compete with other males for mates and territories; size being a critical characteristic in these competitions (Ohlsson and Smith 2001).

Differences in maternal weights without a difference in fetal weights would be expected under mild nutritional deficiencies according to the "fetus as a perfect parasite" hypothesis (Sohlstrom *et al.* 1994). This theory states that the fetus is able to attain more nutrients per unit of body weight than the mother, so that under conditions of energy restriction the fetus will be unaffected while the mother's condition will continually degenerate. Evidence for this theory is inconclusive, however, as it is in my study. Birth weights were significantly reduced in rats eating the lower calorie pollock diet (P) while their mothers maintained body mass, whereas ICN mothers were significantly smaller but supported larger fetuses, as the "fetal parasite" theory predicts.

The effect of maternal nutrition on the growth of fetus(es) depends not only on the degree of undernutrition, but also on the timing of undernutrition and the species (Widdowson 1981). Generally, the longer the gestational period, the more pronounced the effect of undernutrition on the fetus(es). The effects on the rat, with a 21 day

gestation, are therefore relatively minor (Widdowson 1981) and may buffer fetuses and mothers from deficiencies. Even the guinea pig, with a 67 day gestation, is more severely effected by food shortages during gestation than the rat (Widdowson 1981). Reducing a pregnant guinea pig's food during the latter part of gestation can half the weight of her offspring. For an animal such as the Steller sea lion, with a gestation length of over 8 months (Rowlands and Weir 1984), the possible consequences of nutritional deficiencies on her pups could be much more drastic than those I observed with my animal model.

My study focused solely on the effects of undernutrition on the reproductive health of the female rat and her pups. Paternal undernutrition can also affect reproductive events in mammals. Low food availability has been shown to reduce libido, prostate fluid production, sperm counts, sperm motility, and the rate of successful matings (Blank and Desgardins 1984; Abel 1990). For animals that mate only once a year, food shortages during the mating period could seriously affect the next year's pup counts by reducing the number of successful breeding events. However, because the greatest energy investment into reproduction comes from the female and involves much longer spans of investment (Short 1982), food shortages and their effects are more likely to alter reproductive success through the female's contribution.

The Steller Sea Lion

The objective of my study was to evaluate the quality of two different species of fish to gain insight into the nutritional effects of these species on Steller sea lions. A number of my findings have bearing on the hypothesis that diets dominated by pollock have contributed to the decline of Steller sea lions in the Gulf of Alaska.

Concern over the low caloric density of pollock has fueled feeding studies that monitored the body mass of captive Steller sea lions fed pollock and herring (Rosen and Trites 1997; Rosen and Trites 2000a; Rosen and Trites 2000b). These studies found sea lions increased body mass while consuming herring (0.2 kg/day), but lost mass (~0.6 kg/day) while eating pollock (Rosen and Trites 2000b). Additionally, the heat increment of feeding, or the energy used to digest meals, was higher for pollock than for herring (Rosen and Trites 1997). As the welfare of these endangered species must always be considered, the sea lions were not held on the pollock diets long enough to determine whether their loss of body mass would stabilize or continue to deteriorate.

Rats were able to compensate for the difference in caloric content of the fish diets by increasing their food intakes. To date, Steller sea lions have not yet demonstrated that they can increase their intake. This may be a critical difference between the two species of mammals. The effects of switching to a low caloric diet could be drastic if compensation is not an option for sea lions. On the other hand, it could be an ecologically expensive undertaking for Steller sea lions if they can increase their food intake when consuming low calorie species. Steller sea lions may need to consume 35-80% more pollock than herring to compensate for the low energetic density and high heat increment of feeding of pollock (Rosen and Trites 2000b). If the sea lions were to compensate exactly for caloric difference as the rats did, this would mean a 60 % increase in intake based on the proximate analysis of my fish. For an animal that must travel in cold waters to hunt while leaving vulnerable young behind, a 60 % increase in food consumption is quite high. For example, during the 1983 El Nino when food was scarce, Galapagos fur seals (*Arctocephalus galapagoensis*) increased the time they spent

foraging at sea, which resulted in the starvation of their unfed pups during their absence (Trillmich and Dellinger 1991).

Assuming compensation could occur in the sea lions, one must next assume that this would entirely satisfy their requirements and that there are no other differences between the fish. However, the quality of pollock protein and oil seem to be lower than herring, as seen in the slightly reduced growth and reproductive success of the PP rats. Mink fed low fat gadids were able to increase their intake to make up for the low fat content of pollock, but were still unable to attain growth equal to that of mink eating higher fat fish (Leoschke 1961).

Whether or not compensation is realistic may depend on the behavior of the sea lions, and whether they can simply increase their consumption on a single foraging trip, or if the number of foraging trips would have to be increased. During the 1983 El Nino, Peruvian populations of South American fur seals (*Arctocephalus australis*) made longer foraging trips (4 versus 3 days) and more frequent deep dives when prey were scarce (Majluf 1991). During this same El Nino event, California sea lions (*Zalophus californianus)* also increased the length of their foraging trips (1.7 to 3.9 days) and the amount of time they spent at sea (5 % increase over the year before) (Heath *et al.* 1991). Evidence for these adaptations during times of reduced food quality, rather than reduced food quantity, is not available. There is no evidence for reduced maternal attendance or longer foraging periods at sea in regions of Alaska where Steller sea lions are declining (Milette 1999). Here, the quantity of food does not seem to be an issue. Rather, the high abundance of gadids may be the problem. As sea lions are probably opportunistic feeders, it is unlikely that they would forego large quantities of pollock in search of

higher quality prey (Geraci 1975; Milette 1999). Until technology can properly monitor sea lions for prolonged periods of time, as well as monitor exactly what and how much they are eating, these questions will remain unanswered.

The quality of pollock and herring were determined partially through comparisons between the fatty acid and amino acid contents of the fish, and those required by the rat. The specific requirements for these components are known for very few species, and little is known about what is "essential" for the Steller sea lions or other marine mammals. I thus assume that the nutrients that are considered essential for other mammals (ex. linoleic acid) are also those that are essential for the sea lions (Warner and Breuer 1972).

Despite similar litter sizes, fetuses of rats fed pollock were significantly lighter than those fed H, PP, or the ICN diet. In other mammals, low birth weights have been associated with high incidence of mortality and increased risk of infection and illness (Bulik *et al.* 1999). If small pups are able to survive, they may still be at risk of reduced competitive fitness and reproductive success due to smaller skeletal structures, as discussed earlier (McGuire *et al.* 1995; Ohlsson and Smith 2001). Presently, there is no evidence of reduced pup size in Steller sea lions. On the contrary, pups in the declining populations appear to be larger than in the declining populations (Castellini *et al.* 1993; Merrick *et al.* 1995; Rea 1995). However, pup counts have steadily declined on rookeries during the last thirty years (Trites and Larkin 1996). It is possible that either reproduction is failing earlier, in pregnancy (Calkins and Goodwin 1988), or the effects of poor quality food are being felt after weaning in the juveniles who are no longer buffered from nutritional deficiencies by their mothers. Presently, sea lions do not eat a diet of 100 % pollock, so the negative effects of a pollock diet may be partially buffered

by consuming other species of fish. There is evidence that the more heavily a diet is concentrated on pollock, the greater the population decline of the Steller sea lions (Merrick *et al.* 1997).

The impact of fluctuating fish stocks on populations of Steller sea lions is of utmost importance. Difficulties in conducting controlled experiments on Steller sea lions have made it necessary to develop alternative animal models for use in sea lion research. The results of my study indicate that the primary shortcoming of pollock for rats is its low energy density. Additionally, however, there is evidence of a relatively poor protein quality in pollock that, in combination with a low energetic density, may have repercussions on reproductive success. The consequences of these characteristics could ultimately contribute to the documented decrease in Steller sea lion body size (Calkins and Pitcher 1982; Calkins and Goodwin 1988), and may slow the recovery of sea lion populations whose diet is dominated by pollock.

The Rat as a Model for Steller Sea Lions

A number of factors make the rat a valuable animal model for Steller sea lion research. First, it is the most economical model for nutritional studies, compared to alternative models such as harbor seals or mink which are expensive to maintain and care for. Second, rats are the most common model for human nutritional and toxicological investigations, and their physiology is better understood than most alternatives. Finally, reproductive parameters of rats are easily measured because of their short, predictable estrus cycle and gestation period.

However, a major difference between rats and Steller sea lions appears to be the mechanisms by which they regulate food intake. Rats that were fed pollock (P) were able to compensate for the low caloric content of the diet by increasing their food intake, and by doing so, attained body masses similar to those of rats fed herring. Sea lions in captive studies have not demonstrated this ability to increase their food intake (Rosen and Trites 2000b). This has resulted in weight loss in captive sea lions fed pollock and may be the cause of smaller body sizes in post- versus pre-decline animals (Calkins *et al.* 1998; Rosen and Trites 2000b). This may indicate innate differences between sea lions and rats in the mechanisms that control food intake and satiation.

To further assess the value of the rat as a mammalian model for Steller sea lion nutrition research, future studies should confirm whether sea lions are able to increase their intake to compensate for low calorie diets. If sea lions cannot compensate, then the rat may not be the most suitable model for comparative nutritional research.

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APPENDIX 1: PLASMA PROGESTERONE AND CHOLESTEROL LEVELS DURING PREGNANCY

Introduction

A number of parameters were measured in my rats at the time of sacrifice on Day 19 of pregnancy. Although the data from these measurements are complete, I have found that they do not bear directly on the specific questions I posed in my study. For this reason, the blood parameter results are included separately in this Appendix.

Methodology

The methods followed up to this point are described in Chapter II. At sacrifice on Day 19 of pregnancy, blood samples were obtained via heart puncture under halothane anesthetic (Bimeda-MTC, Cambridge, ON) in heparinized, 3 ml syringes and transferred to heparinized vacutainers. Vacutainers were immediately put on ice and within 1 hour, the plasma was extracted and stored at -30°C until analyzed.

Progesterone

Plasma progesterone levels were determined using a radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles). All analyses were performed on the same day, and the intra-assay coefficient of variation was 1.42% at 83 ng/mL, 3.39% at 103 ng/mL, and 2.75% at 117 ng/mL.

Cholesterol

Plasma cholesterol levels were assayed spectrophotometrically using a colorimetric kit (Roche Diagnostics, Indianapolis). All analyses were performed on the same day and the intra-assay variation was 6.3 ± 0.1%.

Results

Plasma progesterone was reduced in the group fed PH (98.2 ng/mL) and elevated in the ICN group (132.7 ng/mL) (F=2.941, n=49, p=0.031) (Figure 8). There was not a significant relationship between plasma progesterone levels and litter size (Figure 9), nor between progesterone levels and the number of corpora lutea.

There was no significant difference in plasma cholesterol between treatment groups, although the rats fed ICN and P had slightly reduced cholesterol levels. The mean plasma cholesterol level was 30.6 mg/dL (Figure 8).

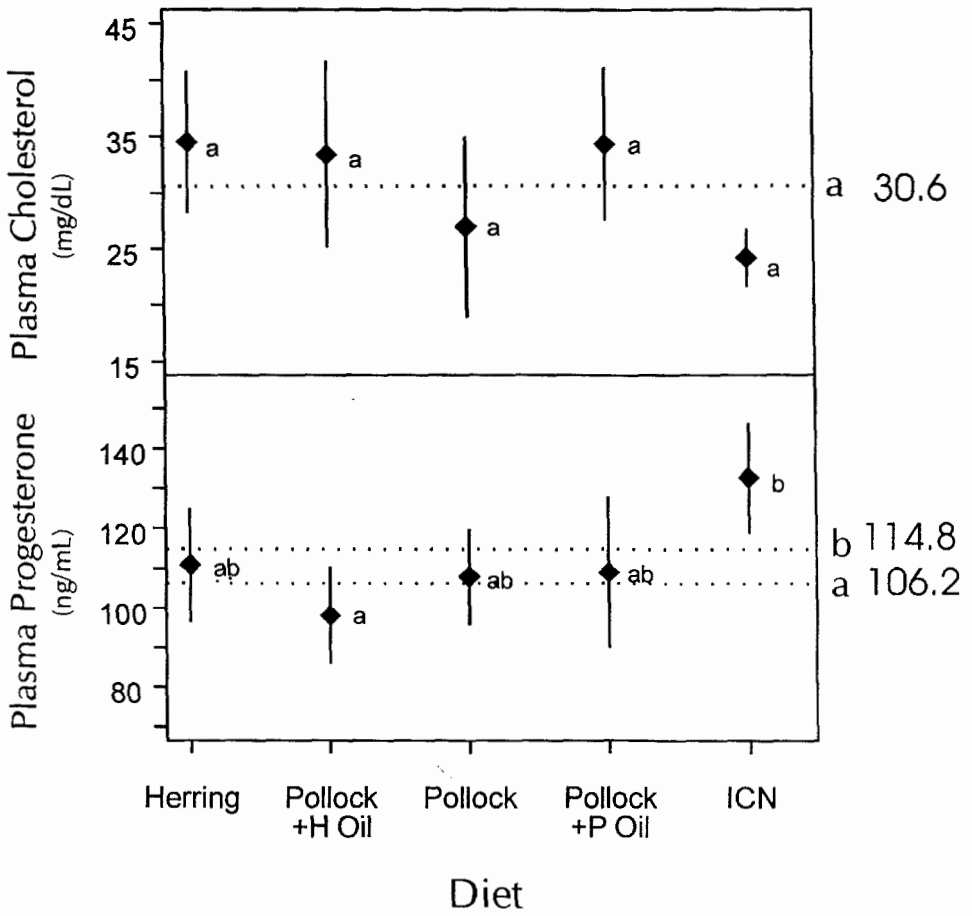

Figure 8. Mean concentrations of cholesterol and progesterone in the blood plasma of rats fed different fish diets or ICN diet. Values are means and 95% confidence intervals (n = 12 per group). Values with different subscripts are significantly different. Values at right are means of statistically similar groups.

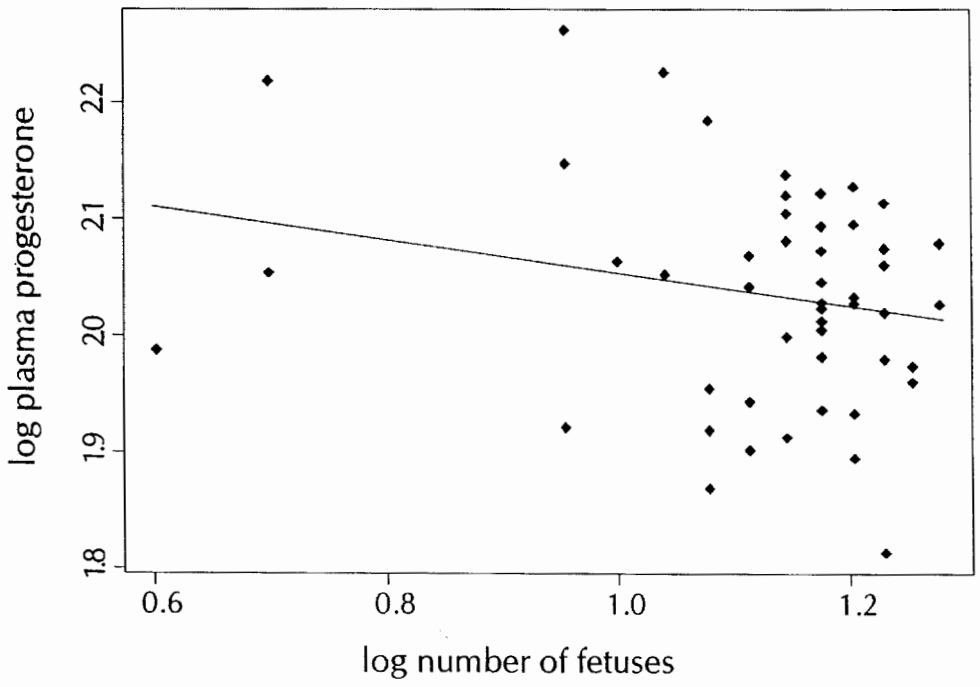

Figure 9. The relationship between plasma progesterone concentrations (ng/mL) and the number of fetuses per female for Wistar rats. Linear regression (log-log): $y = -0.145x + 2.199$, $r^2 = -0.048$, $n = 52$, $p = 0.1298$).

Discussion

Progesterone

Progesterone is a steroid hormone responsible for maintaining pregnancy in mammals (Heap and Flint 1982; Rowlands and Weir 1984). It is secreted by the corpora lutea during early pregnancy, 14 days after which secretion is partially taken over by the placenta (Heap and Flint 1982; Rowlands and Weir 1984). Plasma concentrations rise and remain elevated above non-pregnant levels (Pepe and Rothchild 1973) until approximately 48-72 hours before parturition in the Albino rat, at which time they fall to near pre-pregnancy levels (~12 ng/mL). This decline is believed to initiate parturition (Bartholomeusz *et al.* 1976; Rowlands and Weir 1984).

Circulating progesterone levels ranged between 98.15 ng/mL and 132.66 ng/mL in my study, with those of the ICN group being significantly higher than those fed PH. The mean progesterone level for all groups combined was 111 ± 3.56 ng/mL (mean ± SEM), which is similar to those reported by Pepe and Rothchild (1973) (117.4 ± 12.4) for Sprague-Dawley rats on Day 18 of pregnancy.

Caution must be used in interpreting any differences in progesterone levels as there may have been some error in the assignment of Day 0 of pregnancy in our rats. Progesterone levels change drastically within 24 hours when nearing parturition, and so an overlooked copulatory event during the mating period could have affected my results drastically. Additionally, there is considerable variation between individual rats in the rate of progesterone production, and plasma progesterone levels are typically far above

what is required to maintain pregnancy (Heap and Flint 1982). Differences seen in my study, therefore, may not be of biological relevance.

As progesterone is a key hormone in pregnancy maintenance and is secreted by the placenta in late gestation, one might expect there to be a correlation between circulating progesterone levels and litter size or placental weights. However, there was not a significant relationship between progesterone levels and litter size in my study (Figure 9). This result supports the findings of Bartholomeusz *et al.* (1976) (Bartholomeusz *et al.* 1976) that neither placental weights nor the number of fetuses were related to progesterone levels. Bartholomeusz *et al.* (1976) did find a positive correlation between the number of corpora lutea and progesterone levels, but this was not supported by my study, possibly due to the inherent inaccuracies in the assignment of Day 0 of pregnancy.

Cholesterol

A standard plasma cholesterol level for female Wistar rats approximately 5 months old is 68.2 mg/dL (Lewi and Marsboom 1981), which is 38 mg/dL higher than my mean.

A number of factors could have resulted in the low plasma cholesterol levels in my rats (Subramanian *et al.* 1993). Fish oil has been shown to significantly reduce the blood cholesterol level in rats, due to the high PUFA content of the oil (Cohen *et al.* 1993; Bravo *et al.* 1998; Rabbani *et al.* 1999; Hempenius *et al.* 2000). Male rats fed standard low fat diets (3% fat) had cholesterol levels of 68.04 mg/dL, but when fish oil

was added to the diet in similar quantities (17.5%) as in my study (16.16%), plasma cholesterol was lowered to 39.05 mg/dL (Bravo *et al.* 1998).

There is less of an explanation for why the ICN group had such low cholesterol levels. Fish oil has consistently been shown to reduce plasma cholesterol levels below those of rats fed corn oil, and this has been attributed to the high PUFA content of fish oils (Cohen *et al.* 1993; Prigge *et al.* 1995; Rabbani *et al.* 1999; Hempenius *et al.* 2000). However, linoleic acid, which is the dominant fatty acid in corn oil (Table 6), is known to be a potent hypocholesteremic agent (Grundy 1986; Al-Othman 2000). Additionally, it is known that saturated fatty acids elevate cholesterol while PUFAs lower cholesterol. The effect of saturated fatty acids is stronger than that of PUFAs, and is the primary determinant of plasma cholesterol levels (Hegsted 1991). The ratio of PUFA:saturated fatty acids (Table 6) was highest for the ICN diet, due to higher levels of palmitic acid (16:0) in the fish oils, and so one may expect lower cholesterol levels in the ICN rats.

Despite these arguments, the results of this study still differ from those in the literature, and so error may be an important factor. Similar diets fed under very well controlled studies can yield cholesterol fluctuations differing by as much as 50 mg/dL, and this could be the result of factors other than fatty acids (Hegsted 1991).