Seasonal variation in nutrient composition of Alaskan walleye pollock (*Theragra chalcogramma*) and its effect on the nutritional status of Steller sea lions (*Eumetopias jubatus*)

By

Cynthia Dy Prieto Azana

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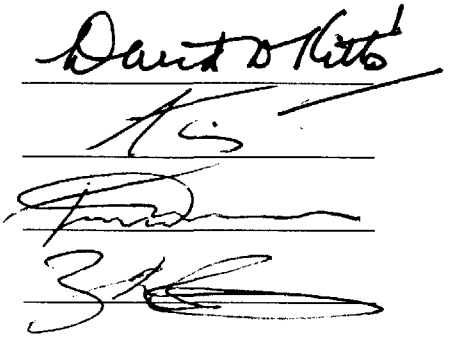

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Abstract

Steller sea lions (*Eumetopias jubatus*) in the Gulf of Alaska declined since the late 1970s. Their population decline might be related to a shift in their diet from fatty, high-calorie fishes such as herring (*Clupea pallasi*) to low energy density fish such as walleye pollock (*Theragara chalcogramma*). I compared the nutritional value of herring with pollock and explored seasonal changes in the nutrient content of pollock. I also compared the nutritional status of three captive Steller sea lions fed pollock and herring. Herring was a more concentrated in dietary lipid ($p<0.001$) and energy source ($p<0.001$) than pollock. The protein of herring was also higher in digestibility ($p=0.015$) than pollock protein, which could indicate that even if ingested energy was equal in both diets, absorbed energy for body functions may be reduced when pollock is eaten. There was little difference in the protein quality of pollock and herring with the exception that valine was more abundant in herring ($p=0.004$). The energy content of pollock changed seasonally, with the peak in energy concentration occurring in the summer and fall (July to November) and then declining over the winter prior to spawning. Captive Steller sea lions lost mass or increased mass at a slower rate on a pollock diet than when they consumed herring, at which time, they all increased in mass. The sea lions had lower levels of plasma cholesterol when fed pollock. Their red blood cells were also more susceptible to oxidation, which corresponded with lower plasma vitamin E levels. These findings suggest that consumption of predominantly pollock has nutritional consequences for the Steller sea lion. Even if they are able to increase their caloric intake to maintain their body mass, Steller sea lions may still be more susceptible to disease originating from oxidative stress.

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Chapter I: Literature Review

1. Marine Mammals in the Gulf of Alaska

The Gulf of Alaska is home to several different marine mammals, which either live there for their entire lives or migrate through the area. For example, many whales and dolphins move into the Gulf of Alaska during the summer to feed and then migrate south during the winter months (Calkins 1986). The Steller sea lion *(Eumetopias jubatus)*, the harbour seal *(Phoca vitulina richardsi)*, the sea otter *(Enhydra lutris)*, and some species of Cetacea make their home in the Gulf of Alaska for their entire lifetime (Calkins 1986). Other pinnipeds, such as the northern fur seal (*Callorhinus ursinus*) and the northern elephant seal (*Mirounga angustirostris)* make seasonal appearances.

Significant population declines have occurred among harbour seals, northern fur seals, and Steller sea lions (Calkins 1986; Trites 1992; Trites et al. 1996; Hobson et al. 1997). The focus of my study is the Steller sea lion, which is the largest of the eared seals and range in the northern regions of the Pacific Ocean (Calkins 1986). The species is named after a German naturalist, who described the sea lions in 1751. Males and females of the species have very obvious physical differences. The adult male can reach, on average, a length of 3 m and a body weight of 681 kg (Winship et al. 2001). Females can reach a length of 2.3 m and 283 kg in body weight (Winship et al. 2001). Breeding season begins in mid-May, when adult sea lions begin to gather on rookeries. Males defend their claimed territory from other males but allow females to move freely in the areas. Pups are born between mid-May and mid-July, but the adult females breed again only ten days after giving birth (Pitcher et al.

1998). Although conception occurs at this time, the embryo does not implant into the uterine wall until the autumn months. Males are generally capable of breeding between the ages of three to seven years old, however they are physically unable to defend a territory until they are more mature, generally at about ten years of age. Females can start to breed at ages three to six and continue to bear a pup each year afterwards.

Steller sea lions feed upon a variety of different prey species, the bulk of which is pollock, followed by squid, pacific cod, herring and capelin (Merrick et al. 1997). The food requirement of the Steller sea lion was predicted at 5% of body mass for 14 year old males and 6% for 22 year old females, with higher food requirements for younger individuals (Winship et al. 2002).

Other pinniped species in the region of the Steller sea lions seem to feed on many of the same prey species and thus, may compete for food. Northern fur seals, Steller sea lions, and harbour seals each have a preference for certain species, but are also opportunistic predators that will take advantage of prey that is abundant (Hobson et al. 1997). Pollock in particular, seems to be a large part of all the diets of these species, but there are preferences with regards to size of the pollock. Steller sea lions appear to prefer juvenile-sized pollock, but are capable of consuming larger fish (Hobson et al. 1997). Northern fur seals and harbour seals consume pollock with a mean fork length that is smaller than what is consumed by the sea lions (Hobson et al. 1997). This common prey species may be the shared link that has led to a decline in the populations of all three pinniped species.

2. Commercial Fishing Methods

It is important to know about commercial fishing methods when doing a study that involves analysis of fish caught by commercial fisheries. While research fisheries may use the same equipment, the focus of a research fishery is to obtain a sample of fish that would be most representative of the population to be studied. In contrast, commercial fisheries are involved in catching fish in a way that is economical and marketable rather than representative. When using commercially caught fish, one should understand this difference.

Fisheries generally classify species of commercial catch into three categories: pelagic species, demersal species, and shellfish. Pelagic fish mainly reside near the ocean surface but may be found anywhere between the ocean bottom and surface (Sainsbury 1971). These would include herring, mackerel, and salmon, among others. They are normally caught with gear that has no contact with the ocean floor. Some species such as herring may gather in shallow waters during spawning and may be caught with gear that has contact with the ocean floor (Sainsbury 1971). Demersal fish live in deeper ocean waters and are closer to the ocean floor. These include pollock, cod, and whiting. Flounder live on or close to the bottom of the ocean while shellfish such as crab, mussels, lobsters, and shrimp, live on the bottom of the ocean.

The type of fishing gear used to make the catch is based on several factors. One is the depth of the waters where the fish will be found. If the net is to touch the ocean floor, the quality of the sea bed becomes a factor. Different gear is used for rough and even sea beds than is used for soft and sandy sea beds. Lastly, different methods of fishing are used for fish that are to be used in bulk for fish meal, animal feeds, etc than for fish that have high individual value. Fish that have high individual value must be caught using methods that

ensure the condition of the fish is of high quality compared to those caught in bulk that may be physically damaged in the nets.

3. Nutrition

My study involved the nutrition of Steller sea lions, so the function of various nutrients must first be explored. The categories of macronutrients are as follows: carbohydrate, protein, and lipid. For the purposes of my study, I mainly focused on protein and lipid since the relative amount of carbohydrate in the Steller sea lion diet is minimal in comparison.

3.1 Protein and Amino Acids

Proteins have multiple functions in the body. First, proteins are necessary for the growth and maintenance of tissues. This includes growth of embryos and developing young animals, as well as replacement of worn out cells such as in the blood, gastrointestinal tract, and skin (Williams 1995). Proteins are required to form the structures of enzymes and some hormones in the body. Enzymes are proteins that act as catalysts for various biochemical reactions in the body. Amino-acid-based hormones, such as thyroid, insulin, and glucagons, are chemical messengers in the body that are released in response to changes in the internal environment of the body. Their release enables the body to respond to the changes and restore the normal conditions (Sizer et al. 1994). Another role of protein is in building immunity against foreign proteins that may enter the body through the production of antibodies. Proteins are also used in fluid and electrolyte balance as well as pH regulation. Proteins can act in this way by either passively attracting water from in or out of the cell or

by the use of transport proteins embedded in the cell membranes, which actively transport ions in and out of the cell that will draw water with them (Zeman 1991; Sizer et al. 1994). The pH balance of the body is maintained by proteins because they can act as buffers to acid or alkaline conditions (Zeman 1991). Lastly, protein can be utilized by the organism for energy in the event that other sources are unavailable (Sizer et al. 1994).

Proteins have a complex, three-dimensional structure. The primary structure of the protein consists of the sequence of amino acids contained within it. This sequence is closely linked to the function of the protein (Lehninger et al. 1993). The secondary structure consists of regularly occurring formations in the chain of amino acids, called a polypeptide, such as the alpha-helix and the beta-conformation. These formations are determined by the interactions of the amino acids in the primary structure. The tertiary structure of the protein is the three-dimensional structure of a polypeptide, which contains within it the secondary structures of the protein. Finally, the three-dimensional polypeptides, which are subunits of a protein, come together to form the quaternary structure of the protein. In my study I will look at both crude protein content of the fish as well as the individual amino acid contents.

Amino acids are important because animals require them in their diets for maintenance, growth, reproduction, and lactation. The metabolism of amino acids by the animal in their gastrointestinal tract can change the composition of the dietary amino acids. Enrichment, impoverishment, or changes of the proportional quantities of the amino acids absorbed into the circulation can occur (Williams 1995). These occurrences can be influenced by the microbial flora in the gut and also by the amount of protein ingested.

Amino acids can also be classified as essential and non-essential. The conventional definition of an essential amino acid is one that "cannot be synthesized by the animal

organism out of materials ordinarily available to the cells at the speed commensurate with the demands for normal growth" (Reeds 2000). These amino acids include histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (Sizer et al. 1994). Non-essential amino acids are ones that can be synthesized by the body at a rate that will meet demands and these include alanine, arginine, aspartate, cystine, glutamate, glycine, proline, serine, and tyrosine (Sizer et al. 1994). The dietary requirements of essential amino acids are difficult to assess due to adaptations that can be made by the animal in times of need such as decreasing urinary nitrogen and increasing digestive efficiency of proteins (Millward 1998). The requirements are generally a function of the animal's metabolic demands and the digestive efficiency of the animal. Little is known about the specific dietary requirements of the Steller sea lion, let alone the amino acid requirements, but there are a few general facts about protein requirements in mammals. One study found a common amino acid pattern in mammalian milk, which could suggest common amino acid requirements of mammalian babies (Davis et al. 1995). Pinniped milk was found to contain large amounts of glutamate, leucine, and proline – approximately summing to 40% of total milk amino acids (Davis et al. 1995). The stage of lactation did not affect the amino acid composition in that study. Another study found that dietary protein deficiency caused delayed maturation and a slowed rate of growth in lab rodents (McAdam et al. 1999). The nestling deer mice of mothers who were fed a low quality plant diet prior to weaning grew at only half the rate of the control group whose mothers were fed high protein cat food. The absolute levels of protein did not affect the nestlings growth as the protein quality did, and they found arginine and valine may have particular importance (McAdam et al. 1999). Protein deficiency during pregnancy was also shown to have detrimental results on the young. Protein malnutrition can

cause decreased placental and fetal growth and cause postnatal damage such as permanently retarded growth and permanent alterations in the structure and function of some organs (Wu et al. 1998). For these reasons, I looked at the protein and amino acid content of the Steller sea lion diet as the protein content and quality may have an effect on their ability to reproduce and also maintain their health as adults.

3.2 Lipids

Fats are nutritionally important because they are the primary form of stored energy in the body. Because they are able to provide more than twice the energy of proteins and carbohydrates, fats provide a compact source of energy, which is valuable in periods of low food availability. Fats are also important as shock absorbers and insulation around vital organs, as structural components of cell membranes, and as a medium for obtaining fat-soluble vitamins A, D, E, and K and essential fatty acids from the diet (Sizer et al. 1994).

There are three main categories of lipids in the body. Triglycerides, also known as triacylglycerols (TAG), represent stored energy and consist of a glycerol molecule with fatty acids esterified to it. Sterols, such as cholesterol are present in most eukaryotic cells and can have various functions in the body such as forming some hormones. Lastly there are structural lipids that form things such as cell membranes. These include phospholipids, which form the phospholipid bilayers of cells and also sphingolipids, which form some structural lipids in the nervous system and are present in small amounts in other membranes (Lehninger et al. 1993).

Aside from their structural function and their function as energy storage, some lipids are biologically active. As mentioned previously, sterols are components of some hormones

such as sex hormones, cortisol, and aldosterone. Eicosanoids are compounds that act in the tissue where they are produced, unlike hormones, and are involved in reproduction, inflammation, fever, pain from injury and disease, blood clotting, blood pressure, gastric acid secretion as well as other functions (Lehninger et al. 1993). Eicosanoids are derived from long-chain polyunsaturated fatty acids, such as arachidonic acid and eicosapentanoic acid. Fat-soluble vitamins also have important functions. Vitamin A is important for night vision and also acts as an antioxidant. Vitamin D has a role in calcium metabolism. Vitamin E is another antioxidant and vitamin K is involved in blood clotting. Essential fatty acids are ones that the body cannot synthesize and are needed for basic functions. These include alpha-linolenic acid, which can be found in foods such as fatty fish, and linoleic acid, which can be found in seeds, nuts, and whole grains (Sizer et al. 1994). Deficiency in these fatty acids is rare but can result in growth retardation, reproductive failure, skin abnormalities, and problems with kidney and liver function (Sizer et al. 1994). Linolenic acid in particular is the precursor for very long chain omega 3 fatty acids, which are important in brain and visual development, reproduction, skin integrity and inflammatory response (Sizer et al. 1994). I looked at the fatty acid composition of the Steller sea lion diet to assess how well their diet meets the needs for the important polyunsaturated fatty acids mentioned here.

4. Nutritional stress in the Steller sea lion

Steller sea lions have been experiencing a population decline in Alaska between Prince William Sound and the far Aleutian Islands since the late 1970s (Trites et al. 1996). One theory is that changes in prey base in these regions have caused nutritional stress on these animals (Trites et al. 2002). The diet of the Steller sea lion in regions of population

decline is composed of primarily walleye pollock (*Theragra chalcogramma*), whereas prior to the decline it consisted of mainly small, fatty, schooling fish such as herring *(Clupea pallasi*) (Merrick et al. 1997). Nutritional stress for the purposes of this study was defined as a decline in health as indicated by decreases in weight or changes in any of the hematological measures taken. Nutritional stress can be caused by the absence of one or more nutrients that are required for optimal health. In the Steller sea lions, small schooling fish may have a greater ability to meet their nutritional needs than pollock.

Of special interest in my study is the energy density of pollock compared to other major components of the sea lion diet, such as herring. Low energy density and poor protein quality can have detrimental effects on the health of sea lions. The poor nutritional quality of their diets may also play a role in the body size reduction of the animals since the 1970s (Calkins et al. 1998). In order to test this hypothesis, I measured and compared the nutrient content of pollock to the nutrient content of herring caught in the same season and year. In addition, captive Steller sea lions were fed diets of exclusively herring or pollock so that their nutritional status on both diets could be assessed.

4.1 Evidence for nutritional stress in the Steller sea lion

Research on the decline of the Steller sea lion has turned its focus to the possibility of nutritional stress in the animals due to a number of physiological changes that have been observed in the population during the years of the decline (Trites et al. 2002). Sea lions in the years of the decline were smaller in mass, length, and girth than sea lions prior to the decline (Calkins et al. 1998). In addition to the size reduction, the body fat was reduced (Castellini et al. 1993) and there was a greater reduction in girth than in length, which is

indicative of nutritional stress in animals (Calkins et al. 1998). Animals may change their reproductive performance in relation to their nutritional status as well. In the Steller sea lion, the rate of pregnancy decreased in lactating females (Pitcher et al. 1998), which implies that lactating females did not obtain enough energy to sustain a pregnancy and nurse a pup at the same time.

4.2 Seasonal variation in energy requirements of Steller sea lions

The energy requirements of the Steller sea lion has been shown to have seasonal variation coinciding with the breeding season, which is from May to July (Winship et al. 2002). During this time, food consumption of male sea lions decreases, as they hold terrestrial territory (Kastelein et al. 1990). The mass of males fluctuate seasonally when they reach sexual maturity and fat stores built outside of the breeding season become especially important. While the female sea lion does not have the same degree of variation in its dietary intake or body mass, the female does have changing nutrient needs due to pregnancy. Typically from January to July, the female carries a growing fetus and is in the later stages of pregnancy. From August to December, most females would be carrying a fetus and suckling a pup as well (Kastelein et al. 1990). Female sea lions with pups have 70% greater food requirements than females of the same age without pups (Winship et al. 2002).

Aside from seasonal changes related to the mating season, variation may originate from changes in environmental temperature as well. When the environmental temperatures were higher, captive sea lions consumed less because the animals required less fat stores for insulation (Kastelein et al. 1990). In low temperatures, food consumption increased in order to build up fat stores. It has also been shown that the more time the sea lion spends in the

water, the more insulation it will need because of the increased body heat losses (Kastelein et al. 1990). Sea lions could very well be affected by seasonal fluctuations in the nutrient content of pollock. For example, an increase in pollock nutrient density during the winter months, when the sea lions are building up the fat stores and have increased energetic needs, could be beneficial to the sea lions because this is the period when their energetic needs are highest. If the opposite were true, the result could be detrimental to the sea lions because the diet would not be adequate to meet the increased energy requirements. For this reason, I explored the seasonal variation in the nutrient composition of pollock.

5. Nutrition Evaluation of Food Stuffs : Proximate Analysis

Proximate analysis consists of determining the amount of moisture, ash, protein, lipid, and carbohydrate in a food item or feed stuff. There are many official methods to accomplish the evaluation of moisture, protein, lipid, and ash, but I will describe only the ones used in my study. Carbohydrate was not analyzed as its content in fish is minimal in comparison with the other components that were measured.

5.1 Moisture

Moisture analysis of foods is important because it is the main constituent of food and allows food processors to have information that could affect the storage and processing of the food item (Bradley 1998). Knowing the moisture content means that other analytical results, such as lipid content, can be expressed as a value based on the dry food. Doing so allows results to be more consistent as many factors can influence the moisture content of samples.

Moisture is also important for pinniped species that live in marine environments because they obtain most of their fresh water from their food (Geraci 1975).

I used the vacuum oven drying method of moisture analysis by placing samples in an oven under reduced pressure. This allowed for more complete removal of water without the decomposition associated with the higher temperatures needed when using a conventional oven (Bradley 1998). This was particularly important with my fish samples because of their high lipid content, which make the fish susceptible to lipid oxidation. Use of a conventional or forced air oven would have altered the chemical composition of the fish and my moisture analyses due to the degradation associated with higher temperatures and exposure of samples to oxygen.

5.2 Protein

I used the Dumas method of protein analysis for the fish samples, which measures total nitrogen in a sample rather than protein. The protein content must be calculated from the nitrogen content using predetermined estimates of the nitrogen content of protein. The factor to determine protein content from nitrogen content varies with different foods. For fish, I used the factor of 6.25, which is the estimate for eggs and meats (Chang 1998) and originates from the fact that most of these proteins contain 16% nitrogen (100/16=6.25) (Chang 1998). Protein content determined by the Dumas method was achieved by combusting the fish samples. Any nitrogen gas that was subsequently released was then quantified by gas chromatography (Chang 1998). The disadvantage to this method is that protein content can be overestimated due to the presence of nitrogen that is not associated with protein in the food.

5.3 Lipid

Crude lipid is often measured using a solvent extraction, such as the Folch's method that I used in my study. Neutral lipids such as triglyceride, wax, and pigment can be extracted from tissues using ethyl ether, chloroform or benzene as solvents (Lehninger et al. 1993). Membrane lipids such as phospholipid are more effectively removed from the sample using polar organic solvents such as ethanol and methanol (Lehninger et al. 1993). Many methods, such as Folch's, include using both types of solvents to remove all lipids from the sample. A common mixture is chloroform and methanol, where the lipids remain in the chloroform layer and the polar molecules, such as protein and carbohydrate, remain in the methanol layer (Lehninger et al. 1993). Since the Folch's method of solvent extraction of lipid is able to remove both polar and neutral lipids, it was used in my study to gain an accurate determination of the total crude lipid of our fish samples.

5.4 Ash

Ash is defined as the total mineral content of a food. This includes trace minerals such as zinc and minerals present in larger amounts such as calcium and iron. I chose the dry ashing method, which involves combusting the samples at high temperatures so that any organic material is burned off, leaving only inorganic minerals behind. This method is advantageous because many samples can be analyzed at once and it is safe in that no reagents are needed and no blanks are required for the analysis (Harbers 1998). There may be loss of some volatile elements, but it is less of a concern because I focused on crude ash rather than trace mineral analysis.

6. Oxidative stress and its health implications

6.1 Oxidative Stress

Oxidative stress is basically a change in the pro-oxidant to antioxidant equilibrium in favor of the pro-oxidants that leads to damage in a biological system (Kehrer et al. 1994). It could also be that the rate of free radical formation is greater than the ability of the cell to transform them into less toxic species (Salem et al. 1997).

Oxidative stress can lead to cellular dysfunction or death and can also produce chemical changes in lipids, protein, and DNA, which can lead to changes in their functionality (Salem et al. 1997). DNA damage can result in mutations, cancer formation, aging, and cellular death and the hydroxyl radical is often the species associated with this type of damage (Acworth et al. 1997). When oxidation affects amino acids in a protein, the protein can lose its functionality especially when the damage happens in a critical part of the amino acid sequence (e.g. the active site of an enzyme) (Acworth et al. 1997). This damage can be a result of oxidation that occurs with aging and can result in a loss of biochemical and physiological function. Oxidized proteins are also more susceptible to proteolysis (Kehrer et al. 1994).

6.2 Free Radicals

Free radicals are species of atoms or molecules that contain one or more unpaired electrons (Ternay et al. 1997). In contrast, a stable molecule has an even number of electrons

in complete orbitals (Pryor 1994). Free radicals can have either a net charge of zero or may carry a charge, usually have very short lifetimes, and decompose very quickly (Ternay et al. 1997). One way that decomposition can occur is through dimerization where two free radicals join their unpaired electrons to form a stable compound. Another way is through disproportionation, which involves the simultaneous oxidation of one radical and the reduction of another to form two stable compounds (Ternay et al. 1997).

6.3 Reactive Oxygen Species

There are a few different species of oxygen radicals that may be present in biological systems. The superoxide anion, O_2^-, is produced by the reduction of O_2. It has been implicated in disease states and its toxicity seems to be related to the Fenton reaction shown below (Ternay et al. 1997). The hydroperoxyl radical, $OH^\bullet$, is more reactive than the superoxide anion and has a larger role in biological damage (Ternay et al. 1997). It is produced during the Fenton reaction (Ternay et al. 1997).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^\bullet$$

Hydrogen peroxide is not a radical, but acts as an oxidant. Though it may react slowly on its own, it can be toxic and result in the formation of hydroxyl radicals when coupled with some metals (Ternay et al. 1997). It also has the ability to diffuse into membranes and lipid deposits (Ternay et al. 1997).

6.4 Lipid peroxidation

Polyunsaturated fatty acids(PUFA) are very susceptible to oxidative damage, particularly those present in structural lipids (Acworth et al. 1997). Polyunsaturated fatty

acids are susceptible to oxidation more so than other fatty acids because of the double bonds in their structure. Lipid peroxidation follows three stages: initiation, propagation, and termination. Oxidation of PUFA, usually by the hydroxyl radical, can result in the formation of a peroxyl radical, which then oxidizes more fatty acids, creating a chain reaction. This is only terminated when either the lipid to protein ratio decreases so the radicals begin to attack protein, or when the lipid radicals encounter an antioxidant (Acworth et al. 1997).

Initiation of lipid peroxidation starts when a carbon attached to a double bond becomes the subject of attack by a free radical (Banks 1997). In biological systems, free radicals can originate from various enzymatic and non-enzymatic reactions (Banks 1997). Lipid peroxidation can also be initiated by iron and other transition metals through a reaction similar to the Fenton reaction in which the metal itself (e.g. Fe^{2+}) reacts with the lipid molecule or the metal can form a complex with hydrogen peroxide that subsequently results in the formation of the hydroxyl radical (Banks 1997). The attack of PUFA by oxidants or free radicals results in the formation of a lipid radical.

After the initiation phase, propagation of the lipid peroxidation process begins and this phase is followed by termination. The initial lipid radical formed by the initiation phase combines with molecular oxygen to form the lipid peroxyl radical (Banks 1997). The peroxyl radical then attacks another polyunsaturated fatty acid, forming lipid hydroperoxides and more lipid radicals (Banks 1997). Iron can also promote the rate of peroxidation by converting lipid hydroperoxides to reactive alkoxyl or peroxyl radicals, which in turn attack another polyunsaturated fatty acid (Banks 1997). These reactions continue in a cycle until the termination phase of the process, in which two radical species combine to form

conjugated lipid dienes, aldehydes, polymers, and hydrocarbons (Banks 1997). The products of lipid peroxidation can be measured to determine the extent of oxidation in a sample.

7. Antioxidants

Antioxidants are essential in controlling the oxidative stress that can cause cellular damage in organisms. The following are some of the antioxidants that aid in fulfilling this function in the body.

7.1 Ascorbic acid

Ascorbic acid is a water-soluble compound that has strong reducing power. This reducing power enables it to be very effective as a free radical scavenger and ascorbic acid works to protect lipids and membranes by scavenging free radicals that may initiate lipid peroxidation (Briviba et al. 1994). It also regenerates lipid-soluble antioxidants such as vitamin E, which get transformed during lipid peroxidation (Briviba et al. 1994). A deficiency in ascorbic acid in the human diet can lead to scurvy, but vitamin C is readily found in many fruits and vegetables so deficiency is rare (Briviba et al. 1994).

7.2 Glutathione

Glutathione is a reducing agent that is made of glutamate, cysteine, and glycine, and is not required in the diet (Briviba et al. 1994). There are two ways in which glutathione works to fight oxidation. First, glutathione can be a substrate for antioxidant enzymes that can reduce hydroperoxides, preventing the accumulation of lipid hydroperoxides (Briviba et al. 1994). Second, it can also react directly with free radicals and in so doing, protect cells

from reactive oxygen species. This causes the formation of GSSG that can be reduced back to GSH with an NADPH dependent enzyme. The GSH to GSSG ratio should be kept high in tissues for it to be effective (Briviba et al. 1994).

7.3 Vitamin E

Vitamin E consists of eight different compounds, which exhibit similar biological activity with the most active of these being alpha-tocopherol (Kijima 1993). The other compounds are alpha, beta, gamma, and delta tocotrienols and beta, gamma, and delta tocopherols (Landvik 1997). It is widely distributed in animals and plants and is especially rich in plant oils (Kijima 1993). Vitamin E in its purified form is either colorless or pale yellow. It is insoluble in water, but soluble in lipids and organic solvents (Kijima 1993).

Vitamin E must be absorbed with dietary fat through the gastrointestinal tract and lymphatic system, and absorption is dependent on the individual animal's ability to do so (Landvik 1997). It is transported in the plasma mainly through low-density lipoproteins and primarily in the form of alpha-tocopherol (Landvik 1997). The exact vitamin E requirements of Steller sea lions are unknown, although it has been shown that animals with high levels of polyunsaturated fats in their diet have great variability in their requirements (Landvik 1997).

Functionally, vitamin E is essential for cellular growth and maintenance of membrane permeability, and is an effective free radical quencher (Lee et al. 2000). Vitamin E has the ability to reduce peroxyl radicals, hydroxyl radicals, superoxide radicals and singlet oxygen in biological membranes (Banks 1997). Vitamin E protects the polyunsaturated fatty acids present in membranes and has also been shown to have a role in mitochondrial function, nucleic acid metabolism, protein metabolism, hormone production, vitamin A protection, and

selenium sparing (Landvik 1997). Vitamin E also appears to be able to modulate heart attack risk by inhibiting smooth muscle cell proliferation (involved in blood vessel wall thickening) and by inhibiting platelet aggregation, adhesion, and platelet release reactions (Traber 2001). Another important function of vitamin E is in the function of the immune system, which deficiency has been shown to depress and supplementation has been shown to stimulate (Meydani 1995). It also has a roles in growth, reproduction, prevention of some diseases, and integrity of tissues (McDowell et al. 1996).

7.4 Others

Other antioxidants include ubiquinone, carotenoids, retinoids, and flavonoids. Ubiquinone is found in soybean oil, meat, fish, nuts, wheat germ, and some vegetables. In humans there are high levels of ubiquinone in the vital organs. In the heart, kidney, and liver, for example, 70-100% of it is present in the reduced form, whereas in the brain and lungs 80% of it is in the oxidized state (Briviba et al. 1994). Retinoids are forms of vitamin A that can be found mainly in vegetables and fruits. Flavonoids are the red, blue, and yellow pigments of plants. They are radical scavengers that work also to chelate iron and inhibit radical-producing enzymes (Briviba et al. 1994).

8. Factors in the variation of fish composition within species

When analyzing the composition of a fish species, it must be understood that results from one study done in a certain region may not apply to fish from other regions and that there is high variability even within a given area. For this reason, in my study, I used a large sample size for the proximate analysis and energy determination to keep the variation in the

results relatively low. Fish caught in different months were also assessed separately because there could be variation between fish caught from one season to the next. There are several factors that can account for the variation in fish composition.

Fish muscle can be classified into two types: white and dark. White muscle is fast muscle used for sudden bursts of speed such as in predation or escape (Love 1988). Dark muscle is slow muscle that is used for continuous swimming (Love 1988). White muscle is less metabolically active than dark muscle and is present in different amounts in different fish. For example, cod, haddock, and whiting are relatively inactive and have more white muscle than active species such as herring, tuna, and mackerel (Love 1988). Dark muscle is different in color than white muscle due to the presence of haem pigments that facilitate the transport of oxygen to the tissues and dark muscle is also higher in the amounts of mitochondria, vitamins, and trace elements than white muscle. Rancidity probably begins in the dark muscle of fish due to the haem pigments and also the greater amount of lipid contained in dark than white muscle (Love 1988). Dark muscle is stronger tasting and probably more nutritious than white muscle due to these inherent differences.

The quantities of these muscle types can also vary within species depending on environmental factors. Water movement is one such factor. Fish that live in an area of high water flow would theoretically have a higher level of swimming effort than fish in still waters and therefore have more dark muscle. This was the case for brown trout that were subjected to an environment that caused them to swim a distance of 1.5 body lengths per second and resulted in an increased proportion of lipid and glycogen content (Davison et al. 1977). Fish in moving waters may in fact be in better condition than fish from still waters.

The pH levels of the water may also contribute to the composition and condition of the fish. Fish are known to die when the pH of their environment falls outside of their range of tolerance. Such changes can cause interference with their metabolic processes especially in the case of freshwater fish and fish in the early stages of development (Love 1988).

The depth of water that fish reside in can affect the composition of the fish and in particular, their fatty acid composition. Medium chain saturated fatty acids and long chain polyunsaturated fatty acids were seen to decrease in the flesh of fish from deep water, but C18:1 increased (Lewis 1967). Another study found that cod caught at 90 to 100m were soft and watery compared to cod caught at closer to the surface at 45m (Love 1988). These changes could improve the buoyancy of fish at greater depths or it could be a reflection of the differences in food supply from shallow to deep water.

Temperature is another factor that affects the fatty acid composition of the fish. As the temperature of the water drops, the proportion of unsaturated fatty acids in the fish increases and saturated fatty acids decrease. This is because the unsaturated fatty acids allow structural lipids to remain flexible in the low temperatures due to the low melting point (Love 1988). This effect is not only seen in fish, but also in zooplankton and algae.

9. Study Objectives and Hypotheses

The overall objective for my study was to determine if there were any differences in the nutritional quality of pollock and herring species and if these differences were sufficient to cause a nutritional stress on Steller sea lions. Of course, knowing the nutrient composition of the fish alone cannot tell the whole story of the possible nutritional stress the sea lions may be under. Variations in nutrient digestion and absorption have direct effects on the

nutritional status of the sea lions, which could be different than would otherwise be predicted from the dietary analysis. For this reason, I studied the effects of an exclusively pollock diet on captive Steller sea lions and compared them to the effects of an exclusively herring diet. I hypothesized that pollock is of less nutritional value than herring, particularly in energy density and that this difference will reflect itself by causing a decline in the health of the sea lions while they are on a pollock diet.

Chapter II: Proximate Analysis of Walleye Pollock – Seasonal Variation and Comparison with Herring

Introduction

Changes in the species composition and the abundance of forage fish in Alaska happened rapidly in the late 1970s (Van Pelt et al. 1997). In particular, walleye pollock appear to have proliferated while herring stocks declined. There also appears to have been a corresponding shift in the diets of marine mammals and birds towards consuming more walleye pollock and fewer fattier fishes such as herring (Merrick et al. 1997). Some have specified that the decline of Steller sea lions is nutritionally based and tied to the apparent dietary shift (Alverson 1992; Rosen et al. 2000a; Trites et al. 2002).

Walleye pollock (*Theragra chalcogramma*) are gadids and occur in the Pacific Ocean from Canada to the Gulf of Alaska, Bering Sea, Aleutian Islands, the Sea of Okhotsk, and Sea of Japan. They live predominantly near the ocean bottom around the continental shelf, but are also found near the surface and in mesopelagic areas of deep waters (OCSEAP 1986). Walleye pollock is an important commercial fish species and is rated one of the highest in total world catches (Janusz et al. 1997).

In addition to humans, pollock have numerous predators in the Gulf of Alaska including several species of fish, seabirds, and marine mammals (Brodeur et al. 1996). The three main predators of pollock in the Gulf of Alaska are Pacific halibut *(Hippoglossus stenolepis)*, arrowtooth flounder *(Atheresthes stomias)* and the Steller sea lion *(Eumetopias*

jubatus) (Hollowed et al. 2000). Cannibalism of juvenile pollock by adults is significant, but is less prevalent in the Gulf of Alaska than in the Bering Sea (Hollowed et al. 2000).

Pollock eggs and larvae can be found in regions of the Gulf of Alaska throughout the year. However, large groups of pollock spawn in certain areas of the Gulf, such as the Sheilikof Strait, in the spring between March and April (OCSEAP 1986; Schabetsberger et al. 1999). At this time, pollock may inadvertently ingest eggs during respiration. Spawning males have been found to have the highest numbers of eggs in their stomachs, mainly due to the amount of time spent in areas of high egg densities (Schabetsberger et al. 1999). Seasonal changes associated with spawning or feeding may result in seasonal changes to the nutritional value of the pollock. Since Steller sea lion nutritional requirements fluctuate during various seasons of the year due to breeding behaviours or water temperatures (Winship et al. 2002), it is of interest to see if there are any seasonal changes in pollock nutrient content.

Pacific herring are a subspecies of Atlantic herring and range from southern California to Korea (OCSEAP 1986). Herring also feed on copepods and euphausiids as do juvenile pollock, and spawn in the Gulf of Alaska only in the spring. They are used commercially for oil, fertilizer, fish meal, bait, and roe and their eggs are harvested. Herring may also be salted and pickled and are the prey of many marine birds, marine mammals, and other fish (OCSEAP 1986).

The shift in the Steller sea lion diet from small schooling fishes such as herring to mainly walleye pollock prompted me to analyze the nutrient content and compare the differences between of these two species. I also examined the digestibility of the protein. In

this way I sought to determine if there were any differences in the ability of each species of fish to meet the nutritional requirements of their predators.

My objective was to identify seasonal and sex differences in the protein, lipid, ash, moisture and energy content of whole walleye pollock. In addition, I wanted to determine how walleye pollock and herring differed. Finally, I wanted to compared the fatty acid profiles, amino acid profiles and protein digestibility to obtain a more complete picture of the nutritional value of pollock and herring.

My main hypothesis of this experiment was that pollock would be of less nutritional value than herring and, in particular, have a lower energy density than herring. Moreover, both species of prey fish would satisfy the requirements for essential amino acids, as animal proteins are generally of good quality. A further hypothesis was that both fish would have a high polyunsaturated fat content, and high content of omega-3 fats in particular would be similar in content, as fish oils are generally rich in these types of fatty acids. I also expected that pollock would have increased lipid content during the spawning season due to the presence of roe in the females and the ingestion of roe by the males. The increase in lipid would correspond with an increase in energy density of pollock from the spawning season.

Materials and Methods

a. Sample preparation

Whole frozen pollock caught in the Bering Sea were obtained monthly in 1998 and 1999 from commercial fisheries (At-Sea Processors Association). A single sample of whole frozen herring was also obtained in 1998 for the purpose of comparison. Pollock and herring were stored either at the Food Science Department at UBC or at the Vancouver Aquarium in a freezer of at least -18°C. Fish were frozen in boxes immediately after being caught and were thawed overnight for grinding. Only fish that were free of major deformities such as missing fins, were selected for grinding and dissection.

The morphology of each fish was evaluated using body weight, girth, length, and weight of gonads. Subsequently the fish were ground in a Hobart Silentcutter, bagged, vacuum-sealed, and frozen at -18°C. Between individual fish, the grinder was washed using a high pressure water spray and thoroughly dried before the next sample. For proximate analysis, small amounts of ground fish were thawed overnight at 4°C and any sample that was not used was freeze dried for subsequent assays.

For the purposes of the study, the pollock were divided into seasons by the month in which they were caught (Table 2.1). Attempts were made at obtaining samples from all months of the year, but as these attempts were unsuccessful, I analyzed samples according to season. For this reason, samples sizes for the seasons were different as I evaluated an equal number of samples from each month that was available. The results from the various months were then pooled according to season. These included winter (January and February), spring (March), summer (July and August), and fall (September, October, and November). Some months were missing due to periods when no pollock fishing occurred. Herring were caught

in November 1998 and were compared only to pollock that were caught in the fall season. Pollock from both 1998 and 1999 were pooled as no significant differences were found between years.

Table 2.1 Summary of walleye pollock used for analysis

Month	Year	Season
January	1998	Winter
February	1999	Winter
March	1999	Spring
July	1999	Summer
August	1999	Summer
September	1998	Fall
October	1998	Fall
	1999	Fall
November	1999	Fall

All fish provided by At-Sea Processors Association and were caught in the Bering Sea

b. Moisture analysis

Moisture determination was done by first pre-drying aluminum pans in a forced air oven at 130 °C for four hours and cooling pans in a dessicator containing silica gel. Analysis of the ground pollock continued by using an analytical balance to weigh 3 to 5 grams of wet sample in dried aluminum pans. The pans with the sample were placed in a vacuum oven at 80°C, 25mmHg, and dried overnight. After removal from the vacuum oven, the samples were cooled in a dessicator. Once completely cooled, the samples were weighed once again on the analytical balance to evaluate moisture loss and total solids. Moisture content (as a percent) was determined by dividing the difference between the initial and final weight of the sample by the initial weight of the sample.

c. Crude Lipid Analysis

Total crude lipid was evaluated by weighing two grams of ground fish in a dried, desiccated Erlenmeyer flask and using the Folch's Double Phase Method (Folch et al., 1957). 50mL of Folch I solution (2: 1 chloroform:methanol) was added to the flask and the mixture was blended. Flasks were then sealed with Parafilm and left overnight. The next day the solution was filtered through fluted Whatman filter paper 4 into a lipid-free glass graduated cylinder. The flasks were rinsed with 10mL of Folch I solution and the rinse solution was then added to the filter. The solid sample left in the filter paper was recovered, stored, and used for protein analysis. Ten milliliters of 0.88% NaCl solution was added to the cylinder, which was sealed, tilted twice, and left overnight. During this time the solution separated into 2 layers. The next day the top layer was suctioned off and 10mL of Folch II solution

(3:48:47 chloroform: methanol: water) was added to the bottom layer in the cylinder that was again sealed, tilted twice and left overnight. The final volume of the bottom layer was recorded the next day. The solvent was evaporated from a known volume of the bottom layer ($CHCl_3$) and the lipid left after evaporation was weighed to determine total crude lipid content.

d. Nitrogen Determination

The total organic nitrogen present was determined using the Leco Method as described by AOAC (AOAC, 992.15, 1995). Air-dried samples (0.25 g) were combusted in an automated Leco FP-328 Nitrogen Analyzer (Leco Corp. Joseph Michigan, USA). The calibration standard was EDTA (9.58% nitrogen). Nitrogen values were multiplied by 6.25 to obtain crude protein values.

e. Crude Ash Analysis

Total ash determination started with preparation of the porcelain crucibles. Crucibles and lids were soaked in detergent overnight, then rinsed and soaked in 3N hydrochloric acid overnight. The crucibles were then rinsed with distilled deionized water and placed overnight in the muffle furnace (lab-heat box type with solid state, vari-watt power level control; Blue M Electric Co., Blue Island IL) at 550°C to remove any contaminants or leftover organic material. Crucibles were removed the next day and cooled in a dessicator before being weighed with the analytical balance. Three grams of wet ground fish was placed in each crucible and heated at 550°C for 32 hours. The crucibles and ash were weighed after being cooled in a dessicator. The percent crude ash was measured in triplicate

and calculated according to weight of crucible and weight of sample before and after ashing, multiplied by 100 percent.

f. Fatty acid profiling

When measuring the fatty acid content of the fish lipid, 10mL of the $CHCl_3$ layer left from the total crude lipid analysis was placed in a fat-free test tube in a 30°C to 40°C water bath to evaporate to dryness. The sample was then cooled to room temperature and 5 mL of 0.5N CH_3OH-KOH was added to the test tube, which was then shaken vigorously. The sample was left overnight at room temperature and 2.5mL of petroleum ether was then added. After the sample separated into two layers, the top layer (consisting of ether and non-saponifiables) was suctioned off and discarded. A drop of 0.4 M HCl and then 5mL of BF_3 were added to the samples and caps were placed loosely on the test tubes to allow gas to escape. The test tube containing the sample was then placed in boiling water and then gradually cooled over 15 to 20 minutes. After the samples reached room temperature, 2 drops of dH_2O were added, followed by 2.5mL of hexane. After the phases separated, the top hexane layer was transferred to an eppendorf tube. Methyl esters were analyzed using a Varian 3700 gas chromatograph (GC-17A; Shimadzu, Scientific Instruments Inc. Columbia, M.D.) equipped with a flame ionization detector and an AOC 1400 auto injector (Shimadzu, Scientific Instruments, Columbus, MD). Samples were injected onto a silicone fused Omegawas TM 320, 30 m x 0.32 mm ID capillary column (Supelco Inc, Bellefonte, PA) with a 0.25 mm film thickness. Helium was the carrier gas. The injector temperature was set at 200°C, with the detector temperature set at 220°C and the column temperature was set at 220°C. The column flow rate was set at 1.9 mL/min. Each sample was analyzed in triplicate

to. A mixture of short chain, medium chain and long chain saturated, monounsaturated and polyunsaturated fatty acids was used as a standard (Sigma Chemical Co., St. Louis MO).

g. Energy Density

Gross energy determinations of pollock and herring were conducted using an adiabatic bomb calorimeter on vacuum dried samples (Department of Animal Science, UBC) according to the Parr method (Parr, 2001). Samples (1.0g) were weighed and individually placed in a sealed bomb calorimeter container, which was sealed with excess oxygen and ignited electrically inside the bomb container. Sample heats of combustion were calculated from the rise in temperature of the water jacket inside the bomb container.

h. In Vitro Digestibility Assay

In vitro digestibility of pollock and herring protein was determined according to the method of Yuan et al. (1991). Ground fish samples were suspended in distilled water and the pH adjusted to 1.9 using hydrochloric acid. Pepsin (porcine stomach mucosa1:10,000-Sigma chemicals) was added to the suspended fish solution, and samples were placed in a shaking 37°C water bath for 30 minutes. Samples were then adjusted to a pH of 8.0 and incubated with pancreatin (porcine pancrease - Sigma, chemicals) and incubated again at 37°C in a shaking water bath. At defined 1 minute intervals, aliquots were removed over a 30 minute period and deproteinized with 20% TCA. Trinitrobenzenesulfonic acid (TNBS) was added to each sample to measure for protein digestion products. Protein digestibility was determined from the initial slope (e.g. 0-10 minutes) using linear regression analysis of

TNBS absorption-time data. Relative digestibility of the different fish protein sources was made in comparison to a casein standard.

i. Amino Acid Analysis

Fish samples (2.0 g) were weighed and refluxed with 6.0 M HCL for 24 hours at 110±1°C under vacuum to obtain complete hydrolysis. Additional samples were hydrolyzed with performic acid to specifically recover cysteine. Moreover, tryptophan was analyzed from samples hydrolyzed with 4.2 M sodium hydroxide for 16 hours at 121 ± 1°C under vacuum. Individual amino acids were quantified using an amino acid analyzer (Pharmacia BiaCore 20) equipped with a cation-exchange column and ninhydrin detection.

j. Statistics

Results were displayed as mean ± SEM. Statistical analysis was done using ANOVA with Tukey post-hoc test for multiple comparisons for results that displayed homogeneity of variances. Kruskal-Wallis and Mann-Whitney tests were done for results that required a non-parametric analysis. Significance was defined as $p \leq 0.05$.

Results

Seasonal differences in pollock

Specific differences in proximate analysis parameters were apparent in the walleye pollock between seasons as indicated in Table 2.2. Of particular interest were the seasonal changes in energy density (Fig 2.1), which was highest in the fall at 5.41 $\pm$ 0.029 kcal/g and decreased during the winter to 5.08 $\pm$ 0.025 kcal/g ($p<0.001$).

Energy density increased in parallel with increases in the lipid content. Lipid content was 17.04 $\pm$ 0.677% in spring and increased significantly to 21.96 $\pm$ 0.479% in the summer months ($p<0.001$). A drop in moisture accompanied this rise from 76.67 $\pm$ 0.201% in spring to 75.88 $\pm$ 0.168% in summer ($p=0.041$). Lipid content was significantly higher in the fall at 21.25 $\pm$ 0.358% than in winter value at 15.44 $\pm$ 0.341% ($p<0.001$).

Protein content in winter was 66.28 $\pm$ 0.513 % and decreased in the spring months to 62.68 $\pm$ 1.077% ($p=0.001$). It reached its lowest levels in the fall at 60.79 $\pm$ 0.442 %, which was significantly, lower than during winter ($p<0.001$).

Ash content was lowest in the summer at 10.59 $\pm$ 0.310 %, which was significantly lower than the value for spring ($p=0.038$). The highest amount of ash was found in the winter, which showed a significant difference from fall ($p=0.003$).

Differences between male and female pollock in their proximate analyses values are indicated in Table 2.3. None of the parameters, which include moisture, protein, lipid, and ash, displayed any distinction between males and females caught in the same season. Energy also did not show significant differences between males and females within seasons. Since no difference was found between sexes, values for males and females could be pooled.

Table 2.2 Seasonal changes in proximate composition and energy content of walleye pollock[1]

Season	Moisture (% wb)	Ash* (% db)	Lipid* (% db)	Protein (% db)	Energy (kcal/g db)	n
winter	77.0 ± 0.1	11.9 ± 0.3	15.4 ± 0.3	66.3 ± 0.5[a]	5.08 ± 0.03	140
spring	76.7 ± 0.2[a]	11.7 ± 0.4[a]	17.0 ± 0.7[a]	62.7 ± 1.1	5.16 ± 0.04	60
summer	75.9 ± 0.2[a]	10.6 ± 0.3	22.0 ± 0.5	62.2 ± 0.5	5.31 ± 0.05	120
fall	74.3 ± 0.1[a]	10.8 ± 0.2[a]	21.3 ± 0.4[a]	60.8 ± 0.4[a]	5.41 ± 0.03[a]	256
ANOVA	$F_{3,576}$ - 68.82	$F_{3,576}$- 6.77	$F_{3,576}$ - 56.03	KW_3 - 82.91	KW_3 - 53.57	
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	

1. Values are presented as mean +/- SEM. a - significant difference with next season (eg. superscript at fall indicates difference with winter)

* values underwent log transformation to find significance. Values with F-ratios underwent a parametric ANOVA.

KW values are Kruskal-Wallis statistics for categories that underwent a non-parametric test. wb = wet basis, db = dry basis

Table 2.3 Sex differences in proximate composition and energy content of walleye pollock[1]

Season	Sex	Moisture (% wb)	Ash* (% db)	Lipid* (% db)	Protein (% db)	Energy (kcal/g db)	n
winter	males	77.2 + 0.2	12.3 ± 0.4	15.0 ± 0.5	66.4 ± 0.8	5.10 ± 0.03	76
	females	76.7 ± 0.2	11.4 ± 0.4	16.0 ± 0.5	66.1 ± 0.7	5.07 ± 0.04	65
spring	males	76.7 ± 0.2	12.1 ± 0.5	17.5 ± 1.1	61.3 ± 1.6	5.11 ± 0.05	33
	females	76.7 ± 0.3	11.2 ± 0.7	16.5 ± 0.7	63.8 ± 1.5	5.22 ± 0.05	28
summer	males	75.9 ± 0.3	11.0 ± 0.5	22.2 ± 0.6	61.0 ± 0.6	5.31 ± 0.06	60
	females	75.9 ± 0.2	10.2 ± 0.4	21.8 ± 0.8	62.3 ± 0.8	5.32 ± 0.09	60
fall	males	75.6 ± 0.2	10.9 ± 0.3	21.4 ± 0.5	61.0 ± 0.5	5.43 ± 0.04	133
	females	74.0 ± 0.2	10.8 ± 0.3	21.2 ± 0.6	60.5± 0.7	5.38 ± 0.04	121
Season	ANOVA	$F_{3,568}$ - 67.37	$F_{3,568}$ - 6.45	$F_{3,568}$ - 55.44	KW_3 - 82.91	KW_3 - 53.57	
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	
Sex	ANOVA	$F_{1,568}$ - 1.69	$F_{1,568}$ - 3.012	$F_{1,568}$ - 0.01	MW_1 - 13013.5	MW_1 - 40067.5	
	p-value	0.195	0.083	0.931	0.411	0.512	
Season-Sex	ANOVA	$F_{3,568}$ - 0.62	$F_{3,568}$ - 1.008	$F_{3,568}$ - 1.22	KW_7 - 86.91	KW_7 - 53.54	
	p-value	0.602	0.389	0.302	<0.001	<0.001	

1. Values are presented as mean +/- SEM.wb = wet basis, db = dry basis * values underwent log transformation to find significance.

No significant differences found between sexes within seasons. F-ratios indicate a parametric ANOVA. KW and MW indicate non parametric tests

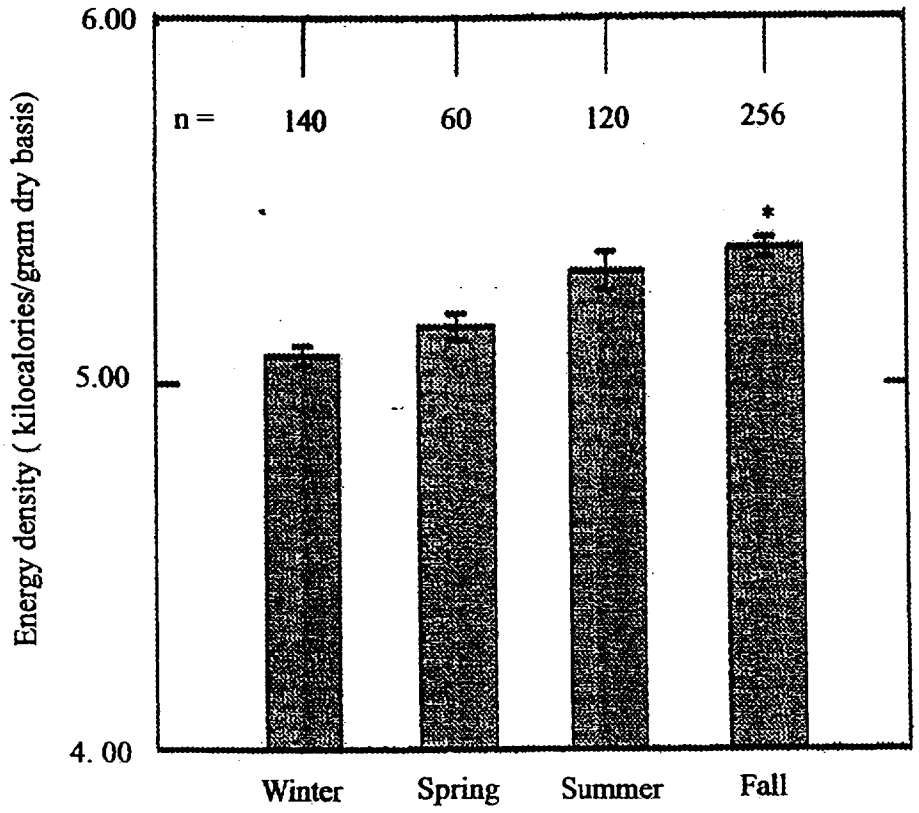

Figure 2.1 Seasonal changes in the energy density of pollock where * indicates a significant difference between fall and winter, $p<0.001$

Seasonal changes in fatty acid composition of pollock

Fatty acid profiles of pollock were affected during different seasons as shown in Tables 2.4 and 2.5. The concentration of omega-3 fatty acid in pollock (Fig 2.2) was highest during the summer, and significantly reduced in the fall ($p<0.001$). Although levels of 18:3n3 and 22:6n3 dropped from spring to summer, the 20:5n3 significantly increase from 14.53% (spring, $p=0.016$) to 17.00% of total fatty acids (summer) making the overall omega-3 fatty acid concentration high in the summer.

This corresponded with the lowest concentrations of omega-6 fatty acids in walleye pollock occurring during the summer months, which were significantly lower from the fall ($p<0.001$) and winter ($p<0.001$) omega-6 fatty acid concentrations.

Table 2.4 Seasonal changes in saturated and monounsaturated fatty acid composition of walleye pollock (% of total fatty acids)[1]

Season	10:0	14:0	16:0	16:1*	18:0*	18:1	n
winter	0.93 ± 0.070	7.45 ± 0.358	24.56 ± 0.911	9.89 ± 0.72[a]	5.15 ± 0.180	22.38 ± 0.639	31
spring	1.13 ± 0.066	7.23 ± 0.708[a]	23.35 ± 0.593	12.43 ± 1.209	4.57 ± 0.216	21.68 ± 0.801	11
summer	1.19 ± 0.073[a]	9.55 ± 0.298[a]	23.41 ± 0.272	11.33 ± 0.329	4.37 ± 0.116[a]	19.56 ± 0.361[a]	58
fall	0.97 ± 0.050	7.21 ± 0.210	24.10 ± 0.290	11.79 ± 0.278[a]	5.06 ± 0.155	22.99 ± 0.311	87
ANOVA							
$F_{3,183}$	3.30	15.98	1.23	8.55	6.87	16.19	
p-value	0.022	<0.001	0.302	0.001	<0.001	<0.001	

1. Values are presented as mean +/- SEM. a - significant difference with next season (eg. superscript at fall indicates difference with winter)

* values underwent log transformation to find significance

Table 2.5 Seasonal changes in polyunsaturated fatty acid composition of walleye pollock (% of total fatty acids)[1]

Season	18:2w6	18:3w3	20:4w6	20:5w3	22:6w3	n
winter	1.29 ± 0.155	0.57 ± 0.096	0.30 ± 0.052	14.39 ± 0.605	13.10 ± 0.527	31
spring	1.13 ± 0.139	0.44 ± 0.080	0.28 ± 0.069[a]	14.53 ± 0.657[a]	13.23 ± 0.851	11
summer	0.93 ± 0.044[a]	0.28 ± 0.041	0.07 ± 0.018[a]	17.00 ± 0.322[a]	12.32 ± 0.372	58
fall	1.39 ± 0.053	0.33 ± 0.032[a]	0.17 ± 0.021[a]	14.59 ± 0.241	11.41 ± 0.367[a]	87
ANOVA						
$F_{3,183}$	8.970	5.046	10.446	12.779	3.058	
p-value	<0.001	0.002	<0.001	<0.001	0.030	

1. Values are presented as mean +/- SEM. a - significant difference with next season (eg. superscript at fall indicates difference with winter)

• values underwent log transformation to find significance

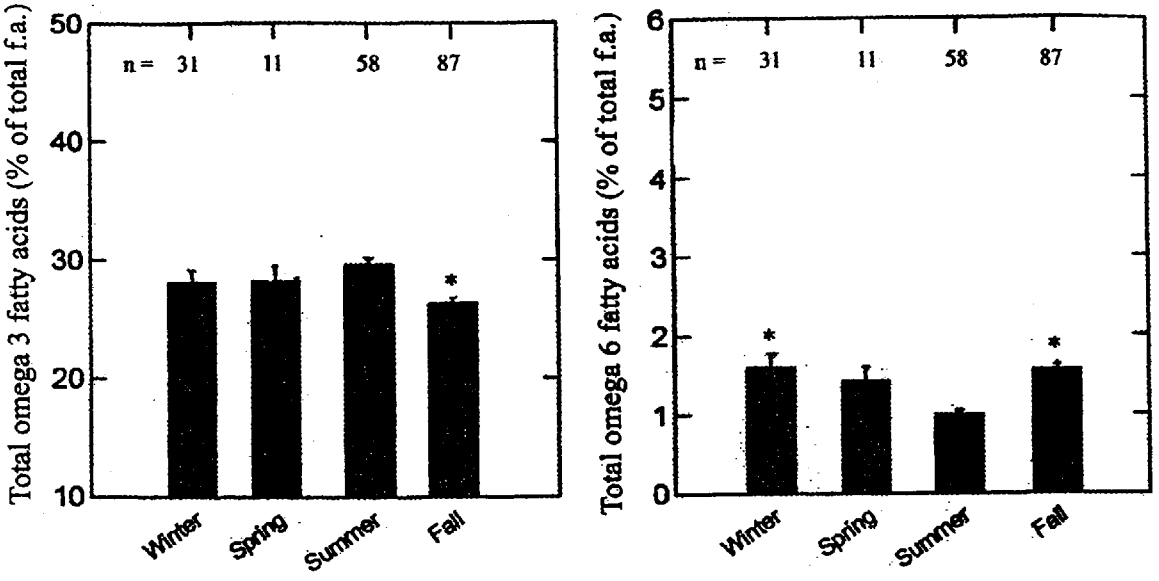

Figure 2.2 Seasonal changes in percentage of polyunsaturated fatty acid content of pollock (*significantly different from summer, $p<0.001$)

Seasonal changes in amino acid composition and protein digestibility in pollock

There were no significant differences in non-essential vs. essential amino acid content of pollock between seasons or in the amounts of individual amino acids as indicated in Tables 2.6 and 2.7. Moreover, no statistically significant changes in the digestibility of the pollock protein (Fig. 2.3) were detected between seasons, although this may be due to the small sample size.

Table 2.6 Seasonal changes in non-essential amino acid composition in walleye pollock expressed as % of total amino acids [1]

Amino Acid	Winter	Spring	Summer	Fall	$F_{3,13}$	p-value
Alanine	7.08 ± 0.212	7.46 ± 0.022	7.00 ± 0.189	7.28 ± 0.172	0.74	0.542
Arginine	6.40 ± 0.189	6.48 ± 0.003	6.82 ± 0.621	6.23 ± 0.128	0.68	0.820
Aspartate	9.87 ± 0.193	10.28 ± 0.136	9.69 ± 0.296	9.98 ± 0.077	1.24	0.335
Cystine	1.01 ± 0.039	0.98 ± 0.002	0.91 ± 0.043	0.96 ± 0.096	0.23	0.871
Glutamate	14.60 ± 0.401	14.55 ± 0.357	14.41 ± 0.244	14.84 ± 0.094	0.68	0.578
Glycine	9.56 ± 0.856	8.18 ± 0.575	8.66 ± 0.379	8.62 ± 0.483	0.70	0.568
Proline	6.33 ± 0.588	4.90 ± 0.062	5.58 ± 0.556	5.71 ± 0.318	1.05	0.402
Serine	5.34 ± 0.172	5.59 ± 0.242	5.46 ± 0.082	5.45 ± 0.067	0.48	0.700
Tyrosine	2.54 ± 0.145	2.91 ± 0.439	3.22 ± 0.462	2.49 ± 0.174	1.53	0.255
n	4	2	4	7		

1. Values are presented as mean +/- SEM. No significant differences found between seasons.

Table 2.7 Seasonal changes in essential amino acid composition in walleye pollock expressed as % of total amino acids [1]

Amino Acid	Winter	Spring	Summer	Fall	$F_{3,13}$	p
Histidine	2.25 ± 0.078	2.24 ± 0.058	2.18 ± 0.048	2.43 ± 0.164	0.669	0.586
Isoleucine	3.80 ± 0.180	3.62 ± 0.017	4.21 ± 0.182	3.88 ± 0.193	1.074	0.394
Leucine	7.78 ± 0.305	8.24 ± 0.257	8.19 ± 0.235	8.09 ± 0.289	0.354	0.787
Lysine	7.29 ± 0.343	7.86 ± 0.122	7.48 ± 0.215	7.60 ± 0.131	0.780	0.526
Methionine	2.16 ± 0.128	2.15 ± 0.124	2.21 ± 0.083	2.33 ± 0.133	0.423	0.740
Phenylalanine	3.51 ± 0.164	3.75 ± 0.197	3.47 ± 0.190	3.56 ± 0.133	0.293	0.830
Threonine	4.97 ± 0.163	5.26 ± 0.168	4.95 ± 0.114	4.96 ± 0.088	0.835	0.498
Tryptophan	1.07 ± 0.064	1.18 ± 0.035	1.01 ± 0.048	1.10 ± 0.142	0.201	0.894
Valine	4.43 ± 0.188	4.38 ± 0.039	4.56 ± 0.183	4.50 ± 0.111	0.185	0.905
n	4	2	4	7		

1. Values are presented as mean +/- SEM. No significant differences found between seasons.

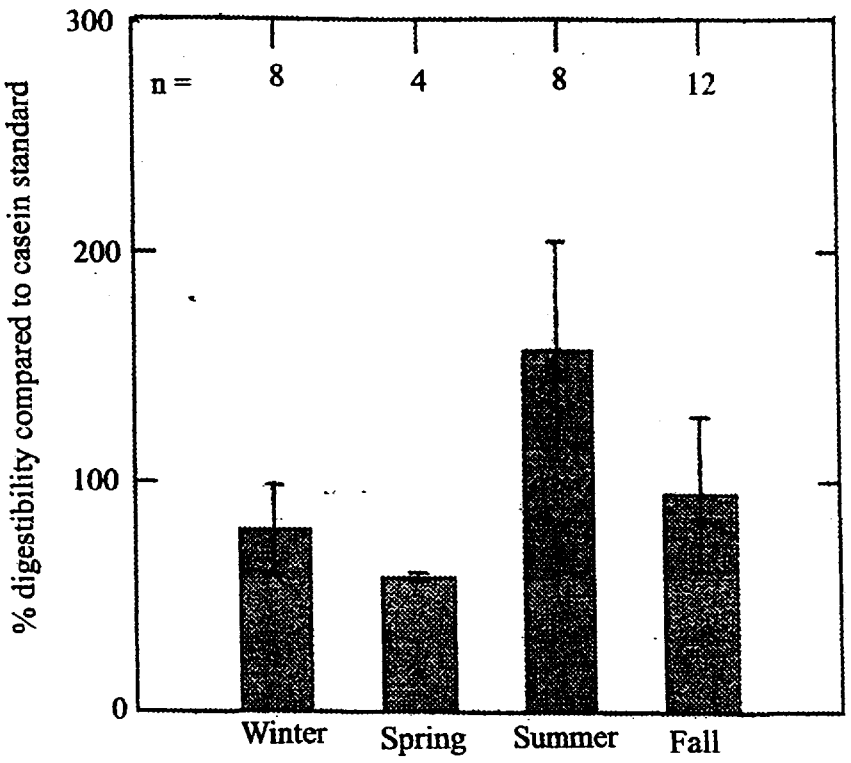

Figure 2.3 Seasonal changes in protein digestibility of pollock ($KW_{3,28} = 8.356$, $p = 0.039$)

Table 2.8 Species differences in proximate composition and energy content. Comparison of walleye pollock vs. herring[1]

Species	Sex.	Moisture (% wb)	Ash* (% db)	Lipid* (% db)	Protein (% db)	Energy (kcal/g db)	n
Pollock	males	74.5 ± 0.2	11.1 ± 0.3	21.4 ± 0.5	60.8 ± 0.7	5.40 ± 0.04	133
	females	74.3 ± 0.2	10.5 ± 0.3	21.2 ± 0.6	58.6 ± 0.9	5.40 ± 0.05	121
	total	74.3 ± 0.1[a]	10.8 ± 0.2[a]	21.3 ± 0.4[a]	60.8 ± 0.4[a]	5.41 ± 0.03[a]	256
Herring	males	65.4 ± 0.7	7.0 ± 0.3	42.3 ± 1.5	39.1 ± 1.1	6.05 ± 0.06	33
	females	64.8 ± 0.6	6.7 ± 0.3	41.2 ± 1.7	39.9 ± 1.2	6.14 ± 0.05	32
	total	65.1 ± 0.5	6.9 ± 0.2	41.6 ± 1.1	39.8 ± 0.8	6.11 ± 0.04	65
Species	ANOVA	MW_1 - 16860.0	$F_{1,315}$ - 140.00	$F_{1,315}$ - 329.08	$F_{1,315}$ - 579.85	MW_1 - 1602.0	
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	
Sex	ANOVA	MW_1 - 48089.0	$F_{1,315}$ - 2.97	$F_{1,315}$ - 0.61	$F_{1,315}$ - 0.01	MW_1 - 50562.5	
	p-value	0.176	0.086	0.436	0.936	0.767	
Species-Sex	ANOVA	KW_3 - 135.98	$F_{1,315}$ - 0.002	$F_{1,315}$ - 0.03	$F_{1,315}$ - 0.34	KW_3 - 102.63	
	p-value	<0.001	0.968	0.871	0.561	<0.001	

1. Values are presented as mean +/- SEM. • values underwent log transformation to find significance, a - values were significantly different between species total values. Values that contain F-ratios, underwent a parametric ANOVA. KW values are Kruskal-Wallis statistics and MW are Mann-Whitney U-test statistics for categories that underwent a non-parametric test. wb = wet basis, db = dry basis

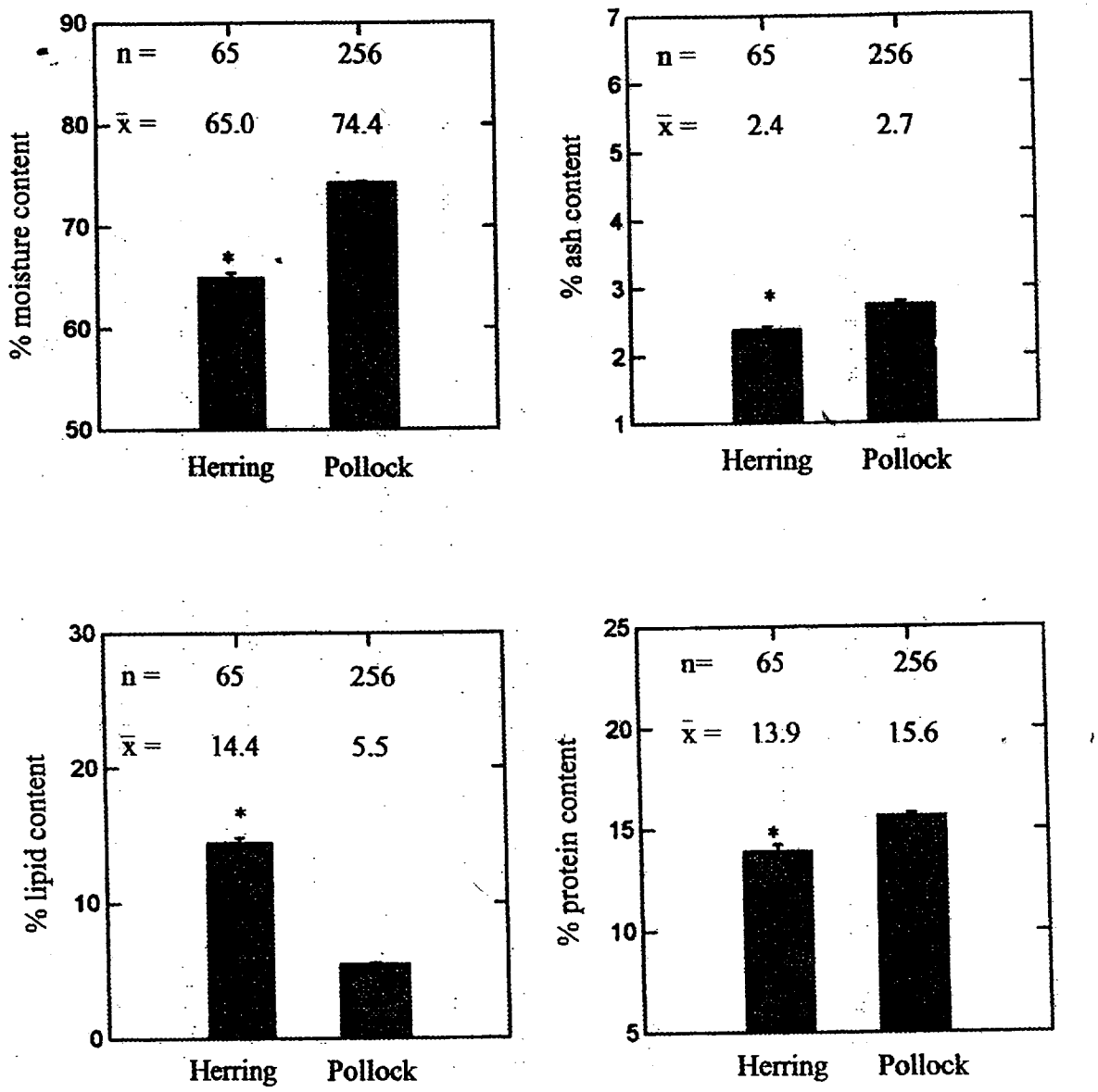

Figure 2.4 Differences in proximate analyses parameters between pollock and herring species on a wet basis (*significant differences, $p<0.001$)

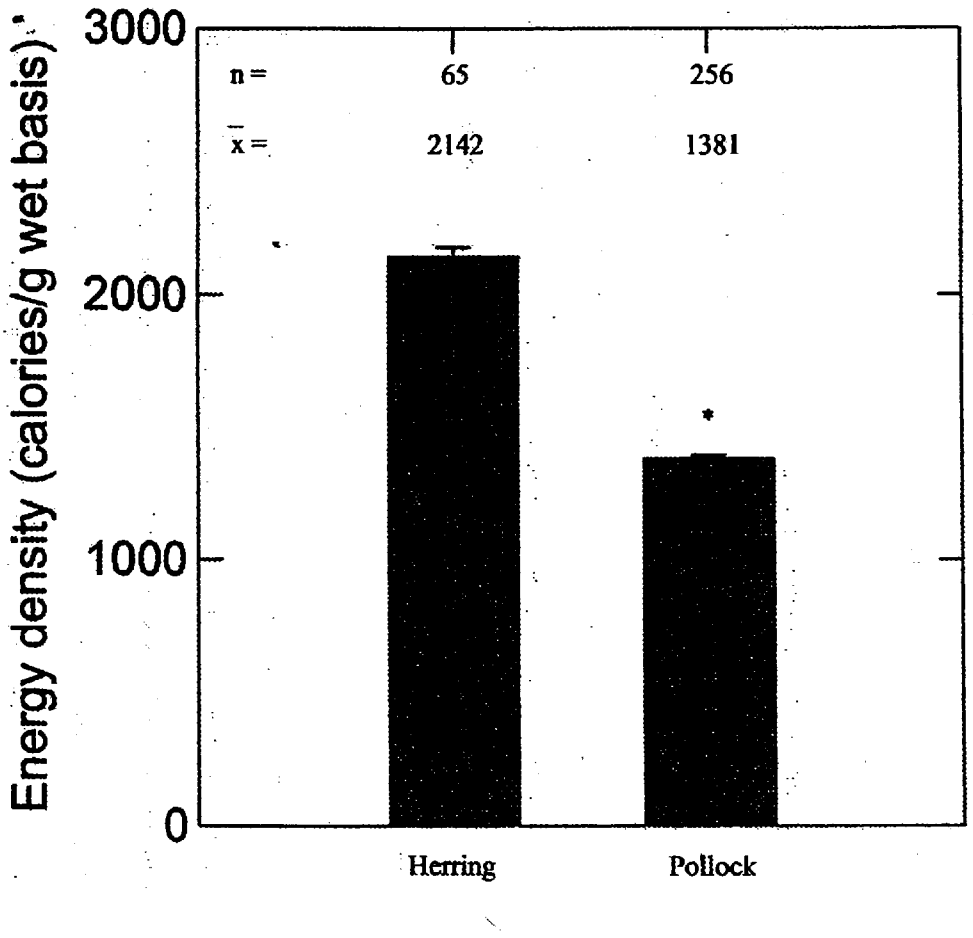

Figure 2.5 Differences in energy density between pollock and herring on a wet basis, *p<0.001

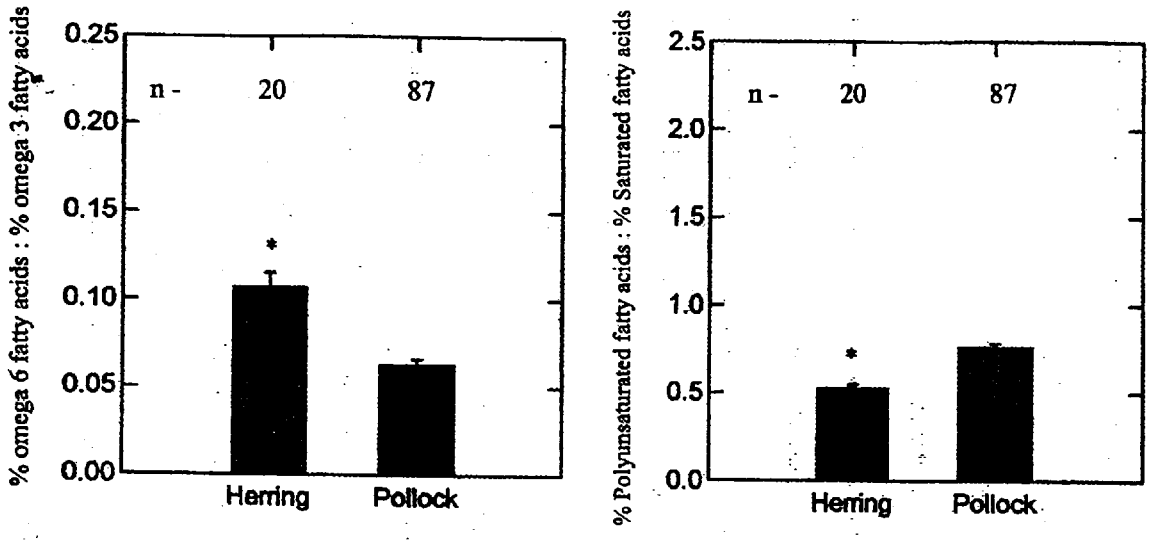

Figure 2.6 Differences in fatty acid composition between pollock and herring (* significant difference, $p<0.001$)

Differences in protein quality and digestibility between pollock and herring caught in the fall season

Serine was higher in pollock than herring ($p<0.001$) and is a non-essential amino acid (Fig 2.8). Valine was higher in herring than in the pollock ($p=0.004$) and is essential (Fig 2.8). There was a slightly higher percentage of total essential amino acids in the herring at 47.8% of total amino acids than in the pollock at 44.9%, but these differences were not considered significant ($p=0.062$) which could be due to a lack of statistical power. Herring protein was found to have an initial rate of digestion at 100.4% of the rate of digestion of casein after five minutes. Pollock protein, on the other hand, had a lower initial rate of digestion compared to casein at 94.8%. These digestibility results were statistically significant ($p=0.015$) based on a non-parametric analysis of variance (Fig 2.7).

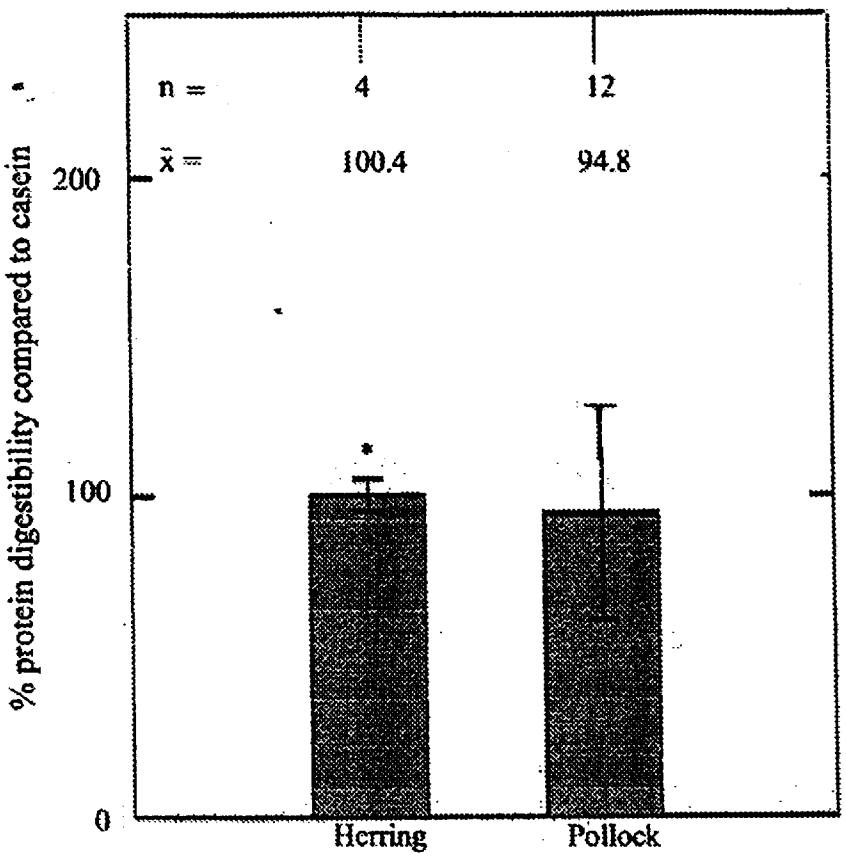

Figure 2.7 Differences in protein digestibility between pollock and herring (*significant difference, $MWU_i = 44.00$, p=0.015)

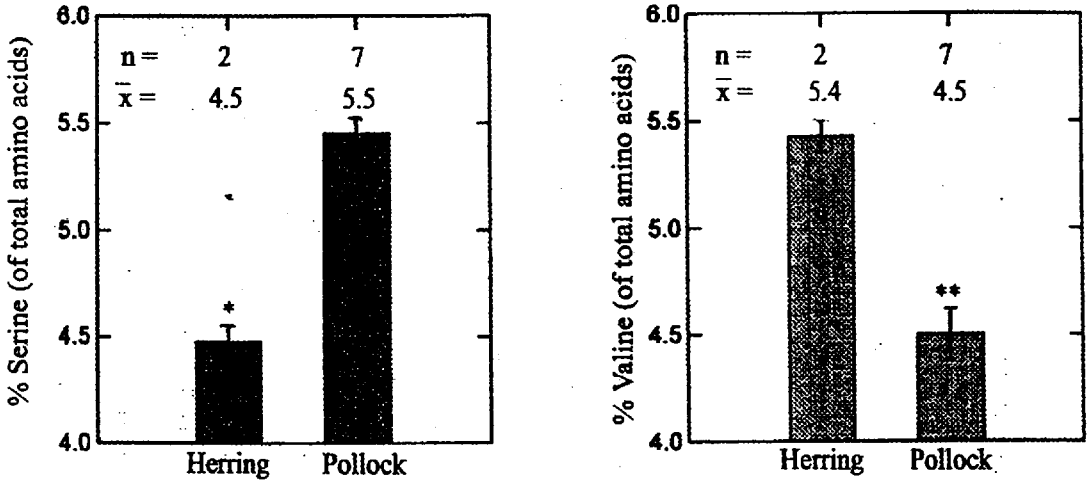

Figure 2.8 Differences in specific amino acid contents between pollock and herring (*p<0.001; **p=0.004)

Discussion

Seasonal changes in proximate analyses and energy density of walleye pollock

The nutrient and energy content of walleye pollock changed seasonally and energy content was lowest in winter and highest in the summer and fall. This loss of energy may reflect changes in eating habits due to fluctuations in prey availability or seasonal behavioral patterns. Winter conditions in the area of the Bering Sea and Alaska are harsh and prey populations may decline to low levels, although there has been no data collected on the specific seasonal changes of food availability in this region (Sogard et al. 2000).

A study on hake (*Merluccius hubbsi*) found that the total nitrogen content as well as the lipid content decreased during the spawning months, which in this case were March and September (Mendez et al. 1997). Hake nitrogen content also remained constant during the winter, whereas lipid content decreased as it was used for energy during times of starvation. This could be comparable to what happens to the pollock during the winter and spring. The energy in pollock may have decreased during the winter due to food shortage. Recovery during spring, when food becomes more available, may have been slow due to the use of muscle lipids and proteins as energy reserves in times of spawning. Slow recovery in spring may also be related to a decrease in feeding during spawning, when starvation appears to occur (OCSEAP 1986; Pedersen et al. 2001) and fish rely on energy stores for survival. Supporting this idea is that pollock are known to be seasonal feeders (Ciannelli et al. 1998).

Steller sea lions, and especially juvenile individuals may target smaller pollock, which would be easier to swallow, and possibly easier to capture than older, larger fish. Juvenile pollock feed at a high rate and grow rapidly during the spring and summer months

in order to survive the winter (Ciannelli et al. 1998). For this reason there may be the same seasonal pattern of body condition for age-0 and juvenile pollock as in adult pollock (Ciannelli et al. 1998). Another study demonstrated that the size and condition of juvenile pollock had a significant correlation with its ability to survive low temperatures and starvation conditions (Sogard et al. 2000). Contradicting Cianelli's results is a study by Paul (1998) who found juvenile pollock increased in energy content in the fall and winter, to a peak level in the spring. Paul's result also contrasts with my data on adult pollock, which had lowest values for winter and spring and the increased energy density occurred in the summer and fall.

Juvenile fish may have lower energy density than adult fish because the ingested energy of a juvenile fish is allocated towards growth rather than storage of lipid (Sogard et al. 2000). This is supported by data in which a previous study done on juvenile age-0 pollock found fish caught in the spring contained 0.955 kcal/g wet mass (Paul et al. 1998) compared to our spring pollock that contained 1.20 kcal/g wet mass. The diet of pollock changes with age. Juvenile pollock consume copepods and euphausiids, but as they grow their diet shifts to fish, such as juvenile pollock, as well as shrimp and crab (OCSEAP 1986). These dietary changes can affect the nutritional value of the pollock. Age-0 pollock exhibit diel migration patterns that enable them to feed and grow efficiently. Cold water reduces the metabolic rate of pollock. Age-0 pollock will swim to warmer waters where prey is more abundant and then retreat into colder waters to use the ingested energy for growth rather than basic metabolic needs (Ciannelli et al. 1998). For this reason, the temperature at which pollock grew optimally decreased as the availability of food decreased (Ciannelli et al. 1998). However, the opposite is true as well. In times when the waters were warmer and the prey

was abundant, optimal growth temperatures would be higher. Strong year classes came from years that were warmer than years where the waters were colder, in addition to other environmental factors (Wespestad et al. 2000). There are no data to state that spawning and seasonal feeding behaviours associated with it occur or do not occur until adulthood in pollock. From the contradictory results of two studies on juveniles, the seasonal variation in energy of juveniles can probably be more closely associated with prey abundance and the diel feeding pattern used as an energy-conserving strategy in juveniles that is non-existent in adults

Other parameters also changed seasonally such as the protein and ash, but it was probably the changing proportion of lipid in the fish that accounts for the changes in energy content, as lipid stores are used up during times of reduced feeding. All of the proximate values in Table 2.1 are expressed on a dry basis, so these changes in proportions are not due to fluctuation in water content. Moisture content also experienced seasonal changes. It was highest in the winter, when lipid and therefore energy content were low, and was lowest in the fall when energy and lipid contents were high. Energy density in my study was expressed as dry weight values, so changes in moisture content would affect how much energy would actually be available to the sea lions when a whole wet fish is ingested. For example, the higher moisture content of the fish in the winter, coupled with the lower energy content in the dry matter of the fish leads to an even greater energy depression than as it may initially appear based on dry matter values. Likewise, the lower moisture content of the fish in the fall months when the energy content is higher on a dry basis leads to a greater amount of energy available to the sea lions compared to winter fish.

Seasonal changes in fatty acids of pollock

Total omega-3 fatty acids were highest in pollock during the summer months. This contrasted with the expectation that long-chain polyunsaturated fatty acid content would be higher in winter to help keep the membranes of the fish more fluid in the colder temperatures. The result may instead be related to the food available to them during these months. In some Kodiak Island bays, pollock consumed primarily chaetognaths and copepods in May, and primarily euphausiids and shrimp in July (OCSEAP 1986). The fatty acid composition of these prey may affect the fatty acids in pollock. However, different species of fish incorporate dietary fatty acids into their cell membranes at different levels (Roy et al. 1999). Dietary fats of carp have been shown to have a limited effect on liver membrane composition due to temperature (Roy et al. 1999) but this was not found in pollock.

The high polyunsaturated fatty acid content of the summer pollock could potentially make their predators, who incorporate these fatty acids into their own tissues, more susceptible to oxidative stress. This is most likely not the case in territorial male Steller sea lions, which are largely fasting during these months, but it may affect the milk of nursing females, and therefore the oxidative status of suckling pups at this time of year.

Seasonal Changes in Protein Digestibility of Walleye pollock

The in vitro method of determining protein digestibility measures the initial rate of protein digestion, which is compared to a standard protein (casein). The products of protein digestion were not removed during the experiment, so the accumulation of digestive products

can inhibit the enzymatic digestion over time. Therefore, only the initial rate of digestion should be considered (Yuan et al. 1991). Protein digestibility in pollock appeared to be higher in the summer than in the other seasons but the difference was not statistically significant due possible to a lack of statistical power. A larger sample should be used to confirm any differences that were seen in my study. Variability in protein digestibility can occur from variations in amino acid composition, although I did not find any seasonal changes in protein quality in my study. Experiments with larger samples of fish for amino acid and protein digestibility should be used to find the true affects of season on protein digestibility in relationship to protein quality.

Implications for Steller sea lions

The seasonal changes in energy density of pollock are disadvantageous to the sea lion when considering the seasonal feeding habits of the species. During the winter, male sea lions build up energy stores that must sustain them throughout the breeding season in the spring. However, I found the lowest levels of energy in pollock occurred during the winter and spring months. The highest energy density as well as the highest level of protein digestibility occurred in the summer and fall. This coincides with behavioral changes of territorial male sea lions, which are fasting during the summer breeding months As well during the winter months, there are increased requirements for fat stores to provide insulation in the colder environmental temperatures for both sexes. The low lipid content of pollock during these months might not allow for the sea lions to ingest the amount of energy needed to meet their needs. It is unlikely that the sea lions would lower their basal metabolic rate in response to the decrease in ingested energy. A 31% drop in basal metabolism was found to

occur in Steller sea lions during periods of fasting, but the same response did not occur during periods of food restriction (Rosen et al. 2002). The extent to which the Steller sea lion diet, in terms of prey composition, changes seasonally is unknown, but generally pinnipeds are believed to be opportunistic predators and take advantage of locally and seasonally abundant food sources (Hobson et al. 1997). Variations in prey consumption would be more likely due to the migratory patterns of fish than selectivity by the animal (Brown et al. 1998). Harbour seals in the United Kingdom have been shown to have significant changes in their diet seasonally. In the spring the seals prey mainly on sand eels, whereas gadids are very dominant in the diet for the rest of the year, being most important during the winter months (Brown et al. 1998). This pattern of feeding generally follows the seasonal availability of the prey species as it most likely would for Steller sea lions as well.

Limitations

Caution should be taken when using the results of my study to support the theory that a pollock diet can be detrimental to the health of the Steller sea lion. While I found significant differences in nutrient content between pollock and herring, it must be noted that my study used samples provided by commercial fisheries. This means that the fish were selected for size by the fishing nets used. A comparison of research and commercial fisheries, however, did find significant correlation between data collected from both groups (Fox et al. 1996). Both groups did use the same gears and methods, which themselves produce bias, such as the mesh size of the net selecting for fish size. In addition, since the nutrient content of fish varies seasonally, I could not apply the pollock versus herring data to other seasons of the year.

Most of the pollock used in my study were large adults, whereas in the wild, sea lions may be more apt to catch juvenile pollock, which would be more similar in size to the adult herring. Pollock being a large and bony fish may be difficult for sea lions, especially juveniles, to swallow when they are full-grown. It is unknown what the differences between juvenile and adult pollock are nutritionally and further study is needed to find any differences. Furthermore, the results could also be affected by the location in which the pollock were caught. Different geographical locations could cause differences in nutrient content due to water temperatures and prey availability. Since my samples came from more than one fishing company, it is possible that various samples were caught at different locations, meaning the differences between seasons could be due to a location difference rather than a seasonal difference. While I did have at least one month's worth of samples from each season, ideally I would have liked to have had samples from every month of the year to assess the pattern of change in nutrient content. There were no pollock caught in the months of April, May, or June and I had no samples from December as well because fisheries do not harvest pollock year round.

Macronutrient content and energy density : walleye pollock versus herring

It is generally known that large demersal and pelagic fish such as pollock are lower in lipid content and therefore energy, than small schooling species, such as herring (Van Pelt et al. 1997). In my study there were significant differences between herring and pollock for all the measured parameters in the proximate analyses. Most importantly is the fact that herring was much more energy dense at 6.1 $\pm$ 0.04 kcal/g dry matter than pollock which had an energy density of 5.4 $\pm$ 0.03 kcal/g on a dry basis. This would be due to the fact that the lipid

content of herring was much greater than pollock, and lipids provide the greatest contribution to energy content (9.2 kcal/g) when compared to protein and carbohydrate (4.2 kcal/g) (Trayhurn 1992). This observation would be further magnified when the data are expressed on a wet basis as herring is considerably lower in water content that pollock (Figs 2.4 and 2.5). It was shown previously that there exists a negative correlation between water content and lipid content in fish (Van Pelt et al. 1997), but it is important to use dry matter to perform bomb calorimetry in order to compare samples where loss of water in samples may occur prior to analysis. Bomb calorimetry is also known to be an overestimate of available or net utilization energy as it measures all combustible material in the sample and does not account for materials that may be indigestible to animals and not available for body maintenance or growth. Pollock is a bonier fish with 10.9 ± 0.23 % of its dry matter as ash, compared to herring, which was found to have 6.9 ± 0.19% ash. This fact probably further contributed to the lower energy density of pollock, but could also make the pollock less palatable than the herring when eaten whole, as with the sea lions. Rosen and Trites (2000b) found that pollock did have a lower dry-matter digestibility compared to herring due to the higher amount of bony material in pollock.

Protein digestibility: Pollock vs. Herring

Tests on pollock for protein digestibility showed that it was less digestible than herring, whose digestibility was on par with casein, the standard used. Coupled with the lower energy density of pollock, the lower digestibility suggests that there would be even less energy available to the sea lion compared to herring.

There are limitations in assuming that in vitro digestibility is equivalent to the gut of an animal, which has interactions of microflora with some of the dietary components. The method of in vitro digestibility used for my study was not specific to the sea lion, which limits its ability to show what would happen to protein in the sea lion's digestive system. However, studies done in vivo with pinnipeds support my in vitro results. For example, Rosen and Trites (2000b) found that the digestive efficiency of Steller sea lions fed herring was greater than when the same sea lions were fed pollock. The dry matter digestibility, which is also known as assimilation efficiency, of herring was also found to be greater than for pollock (Rosen et al. 2000b). This supports my finding that herring protein was more digestible than pollock. Another study done in ringed seals found that the animal's efficiency at assimilating ingested energy also decreased as food quality (defined as energy and lipid) decreased (Lawson et al. 1996). The ringed seals had the highest assimilation efficiency for herring and the lowest efficiencies were found for low energy fish (Lawson et al. 1996). The studies with ringed seals and Steller sea lions did not measure only protein digestibility, but the digestibility of the entire fish. Protein was independently evaluated in another in vivo study done on rats, which found that herring containing diets had a higher protein efficiency ratio than pollock-containing diets (Donnelly et al. 2002).

Implications of the differences between pollock and herring for Steller sea lions

Differences in nutrient content of pollock and herring are fundamental to understanding the plight of the Steller sea lion. Herring stocks make up a lower proportion of the sea lion diet than they once did in the areas of the population decline, while pollock have increased. One theory on why this change has occurred is that long-term climate

changes have affected the abundance of various fish species (Klyashtorin 1998; Benson et al. 2002). Pollock seem to prefer warmer temperatures, with strong year classes generally seen in warmer years (Wespestad et al. 2000). In contrast, herring seem to prefer lower temperatures and strong year classes have been associated with low sea surface temperatures in a study done in Japan (Nagasawa 2001). Regardless of the reason for the prey population shift, the abundance of pollock in the sea lion diet may cause nutritional stress due to the energetic needs of the sea lion not being met.

The lower digestibility of pollock protein may have compounded the lower energy density by having less ingested energy available after digestion and absorption by the sea lion. Animals that consume pollock instead of herring would have to increase their food intake to compensate for the difference in energy density. They also have to compensate for the energy that is not absorbed and for the increased energy they require to digest larger meal sizes (Rosen et al. 1997).

Strictly using the values I found in my study, without considering digestibility of energy, sea lions would have to consume 35.5% more pollock than herring to get the same caloric content. The disparity increases if consideration is given to the amount of energy actually absorbed from the fish and the energy used for digestion (heat increment of feeding). Using previously determined digestive efficiency values of 93.9% for pollock and 95.4% for herring (Rosen et al. 2000b) and the energy lost from the heat increment of feeding (15.7% for pollock and 11.9% for herring) (Rosen et al. 1997; Rosen et al. 2000a), I found that the Steller sea lion would need to consume 60.4% more pollock than herring to obtain the same amount of usable energy. This is close to the value found by Rosen and Trites (2000a),

which found that an average of 56% more pollock than herring would be needed for the sea lions to obtain the same utilizable energy as herring.

Fatty acid profiles

Not only does the amount of lipid in the fish influence nutritional quality, but the specific types of fatty acids present in the lipid are also important. For example, if more saturated fat is ingested in the diet than polyunsaturated fat, then plasma cholesterol may become elevated (Bruckner 1992). Polyunsaturated fatty acids, and in particular, omega-3 fatty acids are widely known to be beneficial to health because of the effect they have on modifying blood lipid characteristics. Long chain omega-3 polyunsaturated fatty acids (PUFA) are instrumental in preventing thrombosis and atherosclerosis and can also increase the proportion of omega-3 PUFA in cardiac phospholipids, which affect the physiological functioning of the heart (Sergiel et al. 1998). Omega-3 fatty acids include the essential linolenic acid, as well as docosahexanoic acid (DHA) and eicosapentanoic acid (EPA). These fatty acids are precursors to compounds that can cause vasodilation and can reduce platelet synthesis, thereby reducing the risk of a heart attack (Bruckner 1992). Omega-6 fatty acids, such as linoleic acid and arachidonic acid are precursors to compounds that can encourage platelet formation and vasoconstriction. Since both omega-3 and omega-6 fatty acids compete for the same biochemical pathway to form respective vasoactive compounds, the ratio of omega-3 to omega-6 in the diet is also important to cardiovascular health.

The herring in my study had a higher ratio of omega-6 to omega-3 fatty acids than pollock. Pollock also had a higher ratio of polyunsaturated fatty acids to saturated fatty acids when compared to herring.

In terms of human health, the lower fat content of pollock as well as the low saturated fat content and high ratio of omega-3 fatty acids to omega-6 fatty acids would be beneficial for cardiovascular health. It is not known however, what the effects of these parameters would be on the Steller sea lions. Sea lions need the high fat content of herring to have sufficient energy stores for breeding and also for protection from the cold water temperatures in Alaska. It is also unknown how prevalent any sort of cardiovascular disease is in the Steller sea lion population. The genetics of each mammal population can make them more or less susceptible to cardiovascular disease when fed cholesterol or saturated fat because of the varying lipoprotein profiles in each species (Kwiterovich 1997). A more important consideration would be the affect on oxidative status of the sea lion.

The greater amount of PUFA in pollock than in herring could also make the sea lion more susceptible to oxidative stress when eating pollock. If the sea lion incorporates these fatty acids into its tissues, those tissues could undergo oxidation whereas saturated and monounsaturated fats would be more resistant to oxidation.

Protein quality

Amino acids are the building blocks of proteins and each amino acid has an important physiological function. Not only is the appropriate amount of dietary protein important, but the protein should also be complete and include all essential amino acids. Deficiency in any essential amino acid could lead to any number of health problems. The needs of Steller sea lions in particular are unknown, so I can only hypothesize about the effects of differences in the amino acid content of their prey. Protein deficiency in laboratory rodents has been shown to delay maturation and slow growth rates (McAdam et al. 1999). Arginine and valine

deficiencies in particular have been implicated in the slowed growth and delayed maturation (McAdam et al. 1999). The lower relative amount of valine I found in pollock compared to herring could explain the growth depression seen in sea lion pups, if they encounter the same problems as the lab rodents. However, the higher serine content of pollock than herring probably has little or no effect on the health of sea lions because serine is a dispensable amino acid that can be synthesized endogenously if no dietary source is available (Reeds 2000).

It is difficult to come to a conclusion about the effects of these differences in amino acids in sea lions, not only because of the lack of data on their amino acid needs, but also because metabolism of proteins in the gut can change the amount of any particular amino acid being absorbed as compared to being ingested. Amino acids can be enriched by an increased absorption rate of a particular amino acid and anabolism. Impoverishment of amino acids from decreased absorption or catabolism, and modification of amino acid composition with increased protein intakes can also occur. Microbes can also alter amino acid absorption. It is unlikely that the small differences in amino acid content between pollock and herring would be of great detriment to the sea lions because my analyses shows that pollock protein does not appear to lack any essential amino acids. More likely, the three dimensional conformation of the protein structure may be what is affecting the differences in digestibility of the pollock and herring proteins.

Summary

The steady decline of Steller sea lions from Prince William Sound through the Aleutian Islands may be related to the shift in their diet from small schooling fish such as herring (*Clupea pallasi*) to primarily walleye pollock *(Theragra chalcogramma)*. I compared the nutritional value of herring with pollock and explored seasonal changes in the nutrient content of pollock. Pollock caught in the winter had the lowest energy density compared to pollock caught in the summer and fall that had the highest energy density ($p<0.001$). Herring had significantly greater energy content than pollock ($p<0.001$) when fish caught in the same season were compared. Herring was lower in ash, moisture, and protein than pollock because a large proportion of the herring mass was made up of lipid, hence the higher energy density. In herring, the proportion of fat that was made up of saturated fatty acids and omega-6 fatty acids was significantly higher than in pollock ($p<0.001$). A significantly higher proportion of the fat of pollock was omega-3 fatty acids compared to herring which had a higher proportion of fat as omega-6 fatty acids ($p<0.001$). There were no major differences in protein quality between the fish, although herring was significantly lower in serine ($p<0.001$) and higher in valine ($p=0.004$) than pollock. Herring protein did exhibit significantly higher digestibility than pollock protein ($p=0.015$). These differences could mean that Steller sea lions that consume primarily pollock are at risk for not meeting their energetic requirements and possibly being under greater oxidative stress due to the higher levels of PUFA in pollock.

Chapter III : Effect of a Pollock Diet on the Nutritional Status of the Steller Sea Lion

Introduction

A shift in diet from fatty fishes to low-fat fishes is thought by some to underlie the decline of Steller sea lions in the Gulf of Alaska and Aleutian Islands since the late 1970s (Alverson 1992; Rosen et al. 2000a; Trites et al. 2002). Diets dominated by any single species of fish can result in intoxication, increased effects of antimetabolites, and malnutrition in pinnipeds, regardless of diet quality Furthermore, some species of fish such as hake and pollock and other gadids can induce anemia in mammals (Geraci 1975) and may cause oxidative stress (Stout et al. 1960; Thompson et al. 1997).

Oxidative stress can cause a number of health problems in animals such as organ failure, carcinogenesis, immune deficiencies and cardiovascular disease. These health problems are caused by damage derived from the products of oxidation reactions in the body. Antioxidants such as vitamin E are therefore very important in regulating the oxidative status of animals. Deficiency of vitamin E in pinnipeds has been shown to cause steatites, muscular degeneration, liver necrosis, and anemia. It can result from dietary inadequacy or destruction of the vitamin E through oxidation (Geraci 1981).

The goal of my study was to evaluate the nutritional status of captive Steller sea lions fed walleye pollock and Pacific herring. Field observations of Steller sea lions in Alaska indicate that body size was reduced during the decline (Calkins et al. 1998)and plasma haptoglobin was elevated (Zenteno-Savin et al. 1997). I therefore measured body size and

hematology and tested the resistance of red blood cells (drawn from the animals while on pollock and herring diets) to oxidation induced in vitro.

The main hypothesis of my study was that the captive sea lions should experience a decline in health (as measured by anemia and weight loss) while fed the pollock diet as compared with the herring diet. The underlying cause for these changes was expected to be due to the greater relative susceptibility to oxidative damage when the sea lions were switched to the pollock diet.

Materials and Methods

a. Feeding Trials on captive Steller sea lions

Three juvenile Steller sea lions, one male (Male 1 – M97KO) and two females (Female 1 – F97HA, Female 2 – F97SI) participated in this study at the Vancouver Aquarium Marine Science Centre. A control diet of herring was administered prior to the experimental feeding trials. The sea lions were allowed to consume the diets *ad libitum*. The trial was done as a crossover with each of the three sea lions eating pollock for six-week treatment periods, followed by six weeks of a herring diet for a control/recovery period. The trial was done twice on each sea lion. The ages of the sea lions ranged between 2.3 to 2.4 years at the beginning of the first pollock trial. Weight, length and girth of the sea lions were recorded during the feeding trials and blood samples were drawn after each feeding period was completed. Blood samples were sent to a veterinary lab for analyses (Central Lab for Veterinarians, Langley, .B.C.), which included hematocrit, hemoglobin concentration, glucose concentration, blood urea nitrogen(BUN), creatinine, serum iron, iron saturation, and total iron binding capacity (TIBC).

b. Sea Lion Plasma Lipid Analyses

Plasma was collected from centrifuged ice-chilled whole blood and analyzed for total cholesterol (Siedel et al., 1983), triacylglycerols (Ziegenhorn, J. 1975:Yuan et al., 1998), and phospholipids (Takayama et al., 1977) using biochemical assay kits (Boehringer Mannheim, Laval, Quebec).

c. In Vitro Forced peroxidation assay for RBC

The method of determining the susceptibility of red blood cells to oxidation was taken from the studies of (Yuan et al. 1996) and (Yuan et al. 2002). Briefly, aliquots (50uL) of packed RBC were diluted into a 10% suspension with 0.9% NaCl-2 mM NaN3 and incubated at 37°C for 5 min. The peroxidizing solution (500uL hydrogen peroxide in varying concentrations, freshly prepared in saline azide) was added to the RBC and the mixture incubated at 37°C for 30 min. The reaction was stopped by the addition of 0.5 mL cold 28% TCA-0.1 M Na-arsenite (BDH chemicals, Poole, England), followed by centrifugation at 12,000 x g for 5 min, at 4°C. A 1.0 ml aliquot of supernatant was assayed with 0.5 ml., 0.5%-2-thiobarbituric acid (TBA, Eastman Organic Chemicals, Rochester, NY), which was freshly prepared in 0.025 M NaOH for the formation of malondialdehyde. Samples were boiled for 15 min and cooled to room temperature. Absorption readings were made at 532 nm to determine the amount of thiobarbituric acid reactive substances (TBARS).

d. Vitamin E Assay of Sea Lion Plasma

Extraction of a-tocopherol was performed according to the method of Desai (1984) with modification. Briefly, 0.25 ml of sea lion plasma was mixed with 0.25 ml of water and 0.5 ml of ethanol. 1 ml of hexane was added and mixture was mechanically mixed for 10 minutes, followed by centrifugation at 4000 RPM for 5 minutes at 4°C (Eppendorf 4050). 0.4ml of hexane layer was then removed to a new test tube and evaporated to dry under nitrogen stream. Residue was then dissolved with 0.1ml methanol. 25 ml of the residue was used for injection. Reverse phase column C18 Luna column (5m, 250'4.6mm, Phenomnex,

Torrane, CA) and isocratic flow with methanol: water = 98/2 (v/v) at 1.5 ml/min was used. Tocopherol was monitored at 292nm (Hoglen and Liebler, 1998). The amount of a-tocopherol quantified according to a standard curve obtained from standard a-tocopherol.

Results

Steller sea lion changes in feeding behaviour

The three sea lions that were a part of the feeding trial increased their food intakes when their diets were switched from herring to pollock (Table 3.1). For example, Male 1 consumed, on average, 7.92 $\pm$ 0.882 kg of herring per day, which was significantly lower than the 11.00 $\pm$ 1.278 kg of pollock he consumed per day ($p=0.05$). Female 1 also increased its food intake from 5.40 $\pm$ 0.261kg of herring to 9.11 $\pm$ 0.887 kg of pollock ($p<0.001$). Female 2 did not significantly increase the mass of pollock she ingested. The daily caloric intake, which was calculated by multiplying the mass of fish eaten per day by the energy density values found in Chapter II, did not differ significantly for any of the animals. I also examined the dietary intake in terms of the percentage of body mass consumed and found Male 1 ($p=0.030$) and Female 1 ($p=0.001$) consumed significantly more pollock as a percentage of their body mass (Table 3.1).

Table 3.1 Changes in eating behaviour in Steller sea lions : Pollock diet vs Herring diet[1]

Animal	Diet	mass of fish eaten (kg)	kcal eaten per day	% of body mass eaten
Male 1	Herring	7.92 ± 0.882[a]	16954 ± 1887	4.11 ± 0.497[a]
	Pollock	11.00 ± 1.278	15191 ± 1765	5.98 ± 0.631
Female 1	Herring	5.40 ± 0.261[a]	11561 ± 558	5.20 ± 0.282[a]
	Pollock	9.11 ± 0.887	12575 ± 1225	8.57 ± 0.874
Female 2	Herring	7.29 ± 0.792	15603 ± 1697	5.23 ± 0.719
	Pollock	8.58 ± 0.796	11851 ± 1099	6.62 ± 0.658
ANOVA (diet)	$F_{1,94}$	13.723	1.507	17.550
	p-value	<0.001	0.223	<0.001

1. Values are presented as mean +/- SEM. significant difference is defined as p<=0.05.

The last week of each trial was used to calculate results. a - values were significantly different between diets

Changes in mass of the Steller sea lions

The masses of the sea lions changed when their diets were switched between pollock and herring as indicated in Table 3.2. For Male 1, the first six weeks on pollock had him gaining mass at a rate of 0.0914 kg/day, but he gained mass at a higher rate of 0.3914 kg/day ($p<0.001$) when he was switched to the herring diet. On the second trial with pollock, Male 1 lost weight at a rate of 0.1683 kg/day, and gained back the mass lost at a rate of 0.8646 kg/day ($p<0.001$) when put back on a herring diet for six weeks. Female 1 lost mass with the first feeding period of pollock at a rate of 0.0394 kg/day and gained mass when switched to herring at a rate of 0.1464kg/day ($p<0.001$). This trend in Female 1 continued into the second pollock trial in which she gained weight at a rate of 0.1629 kg/day. When put back on the herring diet, she continued to gain mass, but at a slightly lower rate of 0.1078 kg/day ($p<0.01$). Female 2 lost mass on both of her pollock trials at a rate of 0.1953 kg/day for trial 1 and 0.0989 kg/day during the second trial. She started to gain mass when switched to herring at a rate of 0.0827 kg/day for trial 1 and 0.1113 kg/day for trial 2. The differences in her rates of mass change were also statistically significant for both feeding trials ($p<0.001$).

Table 3.2 Changes in mass of Steller sea lions : pollock diet versus herring diet[1]

Animal	Diet	Rate of Change in mass (kg/day)	
		Trial 1	Trial 2
Male 1	Herring	0.391[a]	0.673[a]
	Pollock	0.091	-0.168
		$t_{2,61} = 10.76$	$t_{2,65} = 8.01$
		$p<0.001$	$p<0.001$
Female 1	Herring	0.146[a]	0.103[a]
	Pollock	-0.039	0.163
		$t_{2,72} = 6.92$	$t_{2,79} = 2.784$
		$p<0.001$	$p<0.01$
Female 2	Herring	0.083[a]	0.060[a]
	Pollock	-0.195	-0.099
		$t_{2,71} = 13.571$	$t_{2,79} = 8.011$
		$p<0.001$	$p<0.001$

1.Rates were taken from plot of mass over time of trial. a - values were significantly different between diets

A t - test for comparison of two slopes from linear regressions was used to find differences.

Blood and Serum analyses

There was a decrease in cholesterol levels (Table 3.4) of the sea lions while on the pollock diet. There was also a decrease in plasma vitamin E content (Fig. 3.1) for all three sea lions when they consumed pollock. Female 1 had a plasma vitamin E content of 19.5 $\pm$ 1.78 ug/mL while on the herring diet, which decreased to 12.9 $\pm$ 0.30 ug/mL while eating pollock. Male 1 also experienced a drop in vitamin E, from 14.71 $\pm$ 1.10 ug/mL on herring to 10.5 $\pm$ 1.21 ug/mL on pollock. The results for the individual sea lions could not be statistically compared because the sample size was only two for each of the measures. When the results for all three animals were pooled I did find there to be a significant decrease in plasma vitamin E levels when the sea lions were consuming pollock ($p=0.032$, $n=6$).

Table 3.3 Changes in blood and serum analyses in Steller sea lions when diet consists of herring vs. pollock[1]

	Diet	Hematocrit L/L	Hemoglobin g/L	Glucose mmol/L	BUN mmol/L	Creatinine umol/L	Serum Iron umol/L	TIBC umol/L	Iron Saturation %
Male 1	Herring	0.41 ± 0.031	137.0 ± 7.00	8.4 ± 0.2	9.2 ± 2.1	122 ± 10	21 ± 2	90 ± 4	19 + 2
	Pollock	0.42 ± 0.009	142.5 ± 1.50	7.5 ± 0.4	8.9 ± 2.2	113 ± 2	16 ± 3	94 ± 3	15 ± 2
Female 1	Herring	0.38 ± 0.010	130.5 ± 0.500	7.2 ± 0.7	8.2 ± 0.9	107 ± 10	16 ± 3	92 ± 2	15 ± 2
	Pollock	0.42 ± 0.040	140.5 ± 12.50	8.0 ± 0.7	8.1 ± 0.5	98 ± 1	20 ± 4	93 ± 7	18 ± 1
Female 2	Herring	0.47 ± 0.026	163.0 ± 7.00	8.0 ± 1.0	7.7 ± 0.4	100 ± 1	20 ± 3	76 ± 7	21 ± 1
	Pollock	0.47 ± 0.023	160.0 ± 5.00	7.6 ± 0.2	9.1 ± 1.1	96 ± 6	23 ± 1	67 ± 3	26 ± 2
ANOVA	$F_{1,10}$	0.620	0.236	0.140	0.142	1.281	0.079	0.020	0.157
(diets)	p-value	0.449	0.638	0.716	0.714	0.284	0.784	0.891	0.701

1. Values are presented as mean +/- SEM. a - values were significantly different between diets

The last week of each feeding trial was used to calculate results. n=2 for each animal during each feeding trial

Table 3.4 Plasma profiles of Steller sea lions when diet consists of herring vs. pollock[1]

	Diet	Triglycerides mmol/L	Phospholipids mmol/L	Cholesterol mg/dL	Vitamin E ug/mL
Male 1	Herring	0.8 ± 0.15	6.0 ± 0.83	480.8 ± 31.40	14.7 ± 0.78
	Pollock	0.6 ± 0.02	5.5 ± 0.26	327.2 ± 5.14	10.5 ± 1.21
Female 1	Herring	0.6 ± 0.10	6.0 ± 0.38	438.2 ± 26.70	19.5 ± 1.78[a]
	Pollock	0.5 ± 0.04	5.3 ± 0.62	347.4 ± 84.65	12.9 ± 0.30
Female 2	Herring	0.5 ± 0.07	7.2 ± 0.18	514.8 ± 216.14	17.2 ± 0.27
	Pollock	0.8 ± 0.03	7.0 ± 0.30	509.5 ± 5.50	16.5 ± 0.50
ANOVA	$F_{1,10}$	0.072	0.851	3.237	6.231
(diets)	p-value	0.794	0.378	0.102	0.032

1. Values are presented as mean +/- SEM. significant difference is defined as $p<=0.05$.

a - values were significantly different between diets

The last week of each feeding trial was used to calculate results. n=2 for each animal during each feeding trial

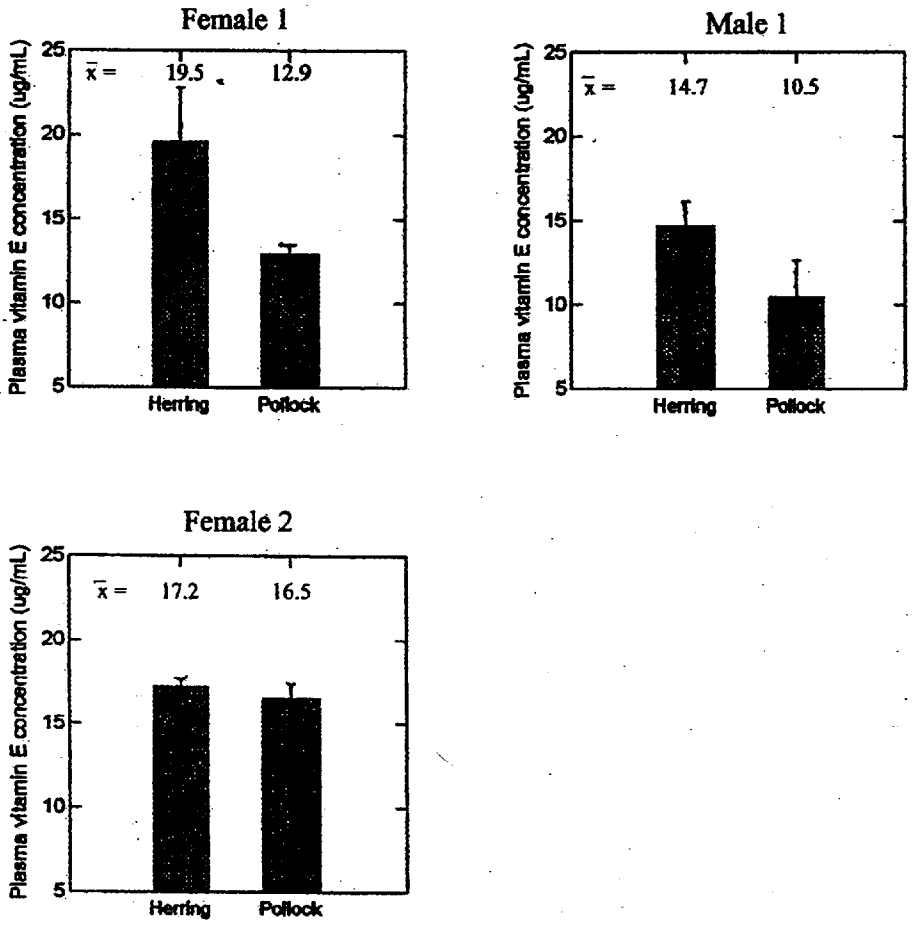

Figure 3.1 Changes in plasma vitmain E concentration in Steller sea lions when switched from herring to pollock diets (n=2 for all measurements)

Forced red blood cell peroxidation assay

All of the Steller sea lion 'pollock' blood samples that were exposed to hydrogen peroxide at varying concentrations experienced an increase in absorbance values at 532nm, an indication of thiobarbituric acid reactive substances, compared to 'herring' blood samples (Figs. 3.2, 3.3, and 3.4). All three sea lions experienced an increase in oxidative stress when fed a diet consisting of pollock.

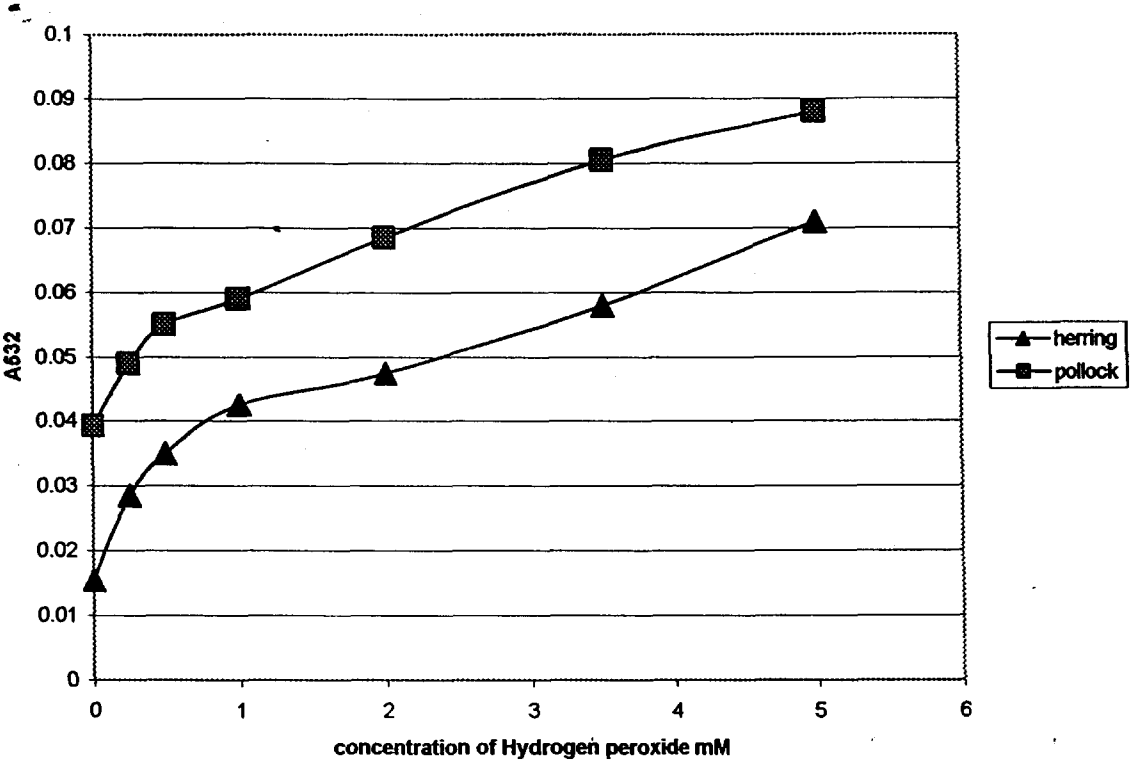

Figure 3.2 In vitro forced peroxidation assay on red blood cells of Male 1

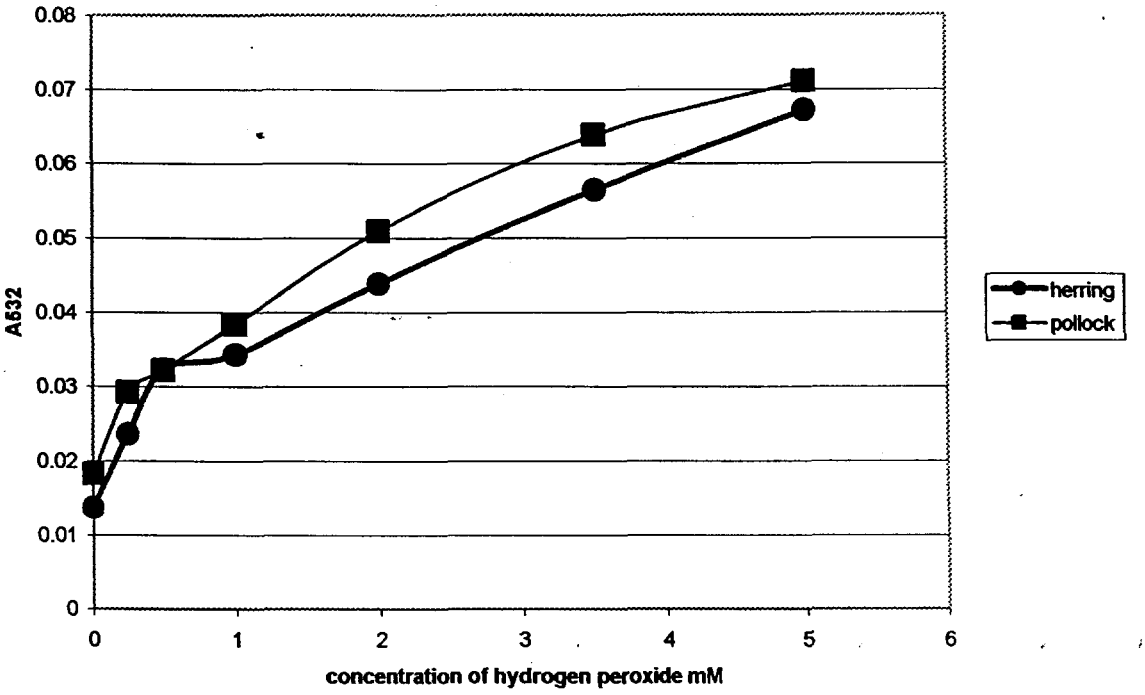

Figure 3.3 In vitro forced peroxidation assay on red blood cells of Female 1

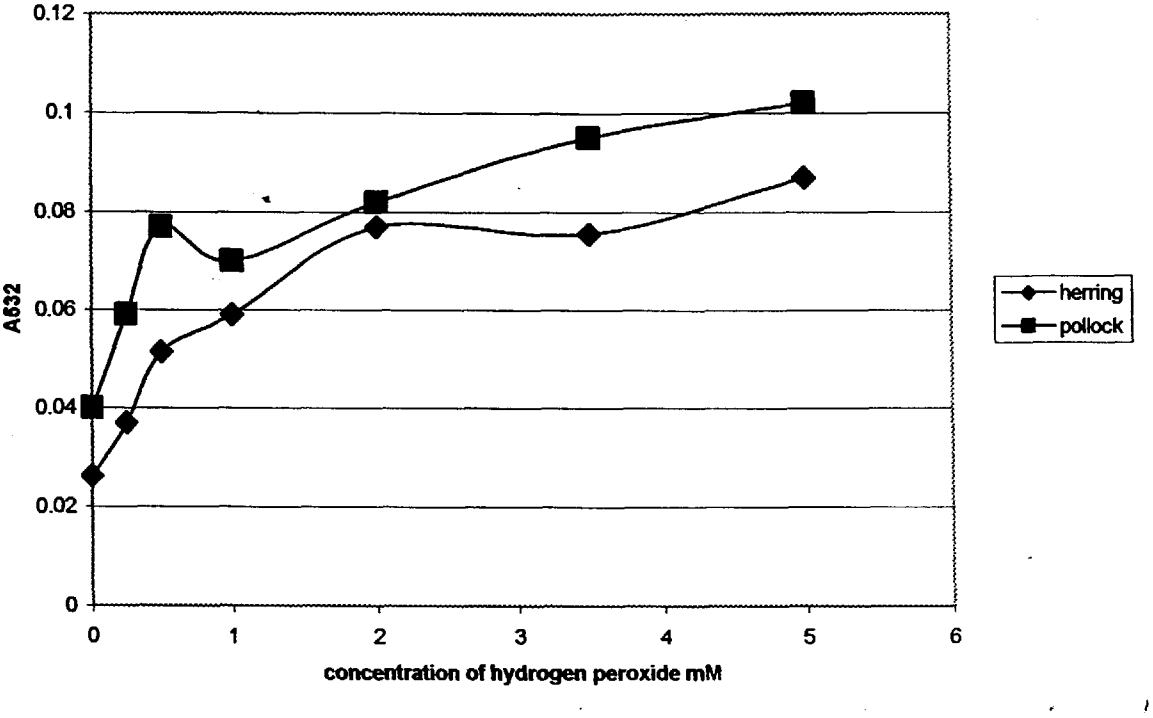

Figure 3.4 In vitro forced peroxidation assay on red blood cells of Female 2

Discussion

Changes in body mass

The size of a Steller sea lion can be an indication of health status (Trites et al. 2002). Generally, animal populations, which have a reduction in body size over a period of time, are thought to suffer from food limitation and malnutrition. This is especially true of animals that undergo a larger reduction in mass than in length, as was found in female Steller sea lions in the Gulf of Alaska (Calkins et al. 1998). A shift in diet from fishes such as herring and capelin, to gadoid fish such as walleye pollock may be the cause of the nutritional stress the sea lions may be under.

The weights of the sea lions in my study indicate that Male 1 and Female 2 clearly had an easier time gaining body mass when fed the herring diet than when fed the pollock diet. Steller sea lions should still be growing until about age 8 for males and age 5 for females (Kastelein et al. 1990; Winship et al. 2001). The weight losses or reduced rates of weight gain that occurred in the animals during the pollock feedings therefore suggest that their nutritional needs were not being met by the pollock as they were on the herring. Although these trials occurred over a short period of time (four six-week periods) these changes in body mass are probably not a result of innate seasonal feeding patterns. Male Steller sea lions do not appear to develop their strong cyclical pattern of feeding until they reach 7 years of age, and females do not appear to have a strong monthly pattern of food intake until they reach 10 years of age (Kastelein et al. 1990). However, other studies have shown some seasonality in juveniles and in adult male sea lions that starting at age 6 between

November and March with little growth from May to September (Winship et al. 2001). This study found that sea lions under the age of 6 grew minimally or decreased in size during the breeding season from June to July (Winship et al. 2001).

My study was done between the end of January to the end of August and crosses between the pre-breeding season period and the breeding season. The first trial in both females began in late winter and into the spring, and the first trial of the male sea lion occurred in the spring months. Both of these periods are thought to be times of increased feeding and growth. The loss of body weight that occurred when the sea lions were fed the pollock diet followed by the weight gain due to the herring diet is probably not an effect that can be attributed to a seasonal change, as I would expect growth to occur throughout this time interval. The second feeding trial of the females occurred from late April into July, and the second trial of the male occurred from early June into late August. During this time, I expected to see a decreased rate of feeding and growth, rather, in Female 2 and Male 1 a similar pattern to the one that occurred in their first feeding trial was observed. In Female 1, however, I saw an increased rate of growth on pollock and a slightly lower rate of growth on herring.

From these results, seasonal patterns of growth probably did not have an impact on the rate of growth of the sea lions as much as the changes in diet did because I would have seen a decrease in growth rates across the trial had it been a seasonal change. My results agree with studies on rats that were fed pollock and herring, where rats fed herring or pollock supplemented with herring oil were heavier than rats fed pollock or pollock supplemented with its own oil (Donnelly et al. 2002).

Changes in feeding behaviour

Instead of looking only at the changes in mass or the amount of food ingested, the influenced of food intake as a relationship with the mass of the sea lion was also examined to correct for increases in food intake related to growth of the animals. Doing so showed Female 1 had a high rate of weight gain on her second trial of pollock, after losing mass during her first trial. This same individual also ingested the greatest amount of pollock for its size (8.57 ± 0.874 % of body mass), which was a 65% increase over the amount of herring consumed. These results indicate that Female 1 was able to increase her caloric intake on pollock as shown in the previous energy analyses of pollock and herring (Chapter II). I estimated that a sea lion would have to consume an average of 60.4% more pollock than herring to get the same utilizable energy. Thus, Female 1 was able to consume enough pollock to meet her energy demand. Both Male 1 and Female 2 also increased the mass of food they consumed when on the pollock diet, but not to the extent that they were able to consume as many calories as they did on the herring diets. Male 1 increased his intake by 45% and Female 2 increased her consumption by 27%, which is clearly not enough to compensate for the lower energy density and digestibility of pollock.

The increased amount of food ingested is in contrast to the study by Rosen and Trites (1999) in which other young Steller sea lions fed a low energy diet of squid did not eat more to compensate decrease in dietary energy density. Another study done with pollock and herring also found that the sea lions did not increase their food intake in relation to the lowered energy intake (Rosen et al. 2000a). However the sea lions were only fed pollock for periods between eleven and twenty-four days (Rosen et al. 2000a), which may not have been enough time to see a significant increase in food intake or may not have been enough

time for the sea lions to adapt to the new food source. The same may be true for the study using squid, where the sea lions were on an ad libitum diet of squid for fourteen days (Rosen et al. 1999).

Time may be needed for the sea lions to physically stretch their stomach capacity to accommodate the increase in food ingested, although palatability of the food given may also be an issue. Rats fed pollock or herring have been shown to increase their consumption of low energy diets to acquire the same amount of energy as rats on the high energy density diets (Donnelly et al. 2002). However, the rats only had to consume 10% more due to the dilution of the fish with other feed ingredients.

The larger amount of pollock eaten by the sea lions in my study does come at an energetic cost. The heat increment of feeding, which is a measure of the cost of processing food, increases as meal size increases (Rosen et al. 1997). In addition protein has the highest energetic cost of digestion and lipid has the lowest (Rosen et al. 1997). Pollock would therefore increase the heat increment of feeding in sea lions due to the larger amount of food ingested to meet metabolic demands, and the higher proportion of protein to lipid than in herring. The Steller sea lions fed pollock in previous studies lost mass and had depressed resting metabolic rates due to the lower amount of energy in the pollock diet (Rosen et al. 1999; Rosen et al. 2000a). These sea lions had a higher rate of weight loss than the animals in my study because they did not increase their dietary intake of the low-energy diet. A more recent study found that Steller sea lions that were fasted had depressed resting metabolic rates, but that sea lions whose food (herring) was restricted did not experience this depression (Rosen et al. 2002).

A bioenergetic model for the Steller sea lion showed that the energy requirements of males and females increased during the winter and spring, even between 2 and 3 years of age (Winship et al. 2002). The sea lions in my study were not tested for changes to their metabolic rates, so it is not known what their metabolic needs were. However, it is clear that they were not adequately met by the pollock diet. Steller sea lions in the wild may have increased needs in comparison to captive animals due to increased activity or time spent in the water, so the effect of eating pollock may be magnified in wild specimens that have to expend more energy in the foraging effort.

Winship et al. (2002) estimated that 3-year-old male sea lions required 21 $\pm$ 5.0 kg of food and females required 17 $\pm$ 3.8kg of food per day in February. This is calculated as 11% of body mass for both males and non-pregnant females using values for predicted mass (189 kg for males, 156 kg for females without a fetus) (Winship et al. 2001). However, the captive animals in my study only ate in the range of 5.40 kg to 11.00kg for both herring and pollock. These values are calculated as in the range of 4.1% to 8.6% of their body masses.

Plasma cholesterol

The sea lions in my study did not exhibit changes in many of the blood analyses that were examined. There was a decrease in plasma total cholesterol for all three animals (32% decrease in Male 1 and 21% decrease in Female 1), as well as a decrease in plasma vitamin E levels when fed pollock. The decrease in plasma total cholesterol was probably due to the higher level of polyunsaturated fat in pollock than in herring (Chapter II, Fig. 2.6). Diets supplemented with menhaden oil, another marine oil which is high in omega 3 fatty acids, caused plasma total cholesterol to decrease in hypercholesteremic chicks and rats (Castillo et

al. 1999; Yuan et al. 2002). In humans high levels of plasma cholesterol and specifically low-density lipoprotein cholesterol, is a major factor in cardiovascular disease.

The effect of cholesterol and incidence of cardiovascular disease is unknown in sea lions. However, cholesterol can have a stabilizing affect on cell membranes by protecting against lipid oxidation. A study done in Japanese quail showed that diets high in cholesterol and saturated fat increased the cholesterol levels of tissue membranes as well as plasma cholesterol (Yuan et al. 1999). While the increase in plasma cholesterol caused the deposition of plaques in the arteries, the increased cholesterol in tissue membranes was associated with greater resistance to peroxidation of liver tissues. If this is correct, then an increased susceptibility to oxidation of the sea lions would occur when they are fed pollock diets due to decreased cholesterol in cell membranes.

Oxidative stress and the role of Vitamin E

I found increased susceptibility to oxidation in the forced peroxidation analyses for all three sea lions that were fed pollock. This may have been due in part to lower levels of cholesterol, but lowered vitamin E levels were probably the primary cause of oxidative stress. Vitamin E is an antioxidant, and the decrease in serum vitamin E for all three of the sea lions may account for the lower resistance to oxidation of their red blood cells.

Oxidative stress can result from the formation of free radicals in biological systems when an animal suffers environmental stress. Damage from oxidation can result in a reduced growth rate, fertility, carcinogenesis, immunodeficiencies, and neurological damage in mammals (Vichnevetskaia et al. 1999). Animals require antioxidants, such as vitamin E and vitamin C in their diet to combat oxidative damage. Vitamin E protects cell membranes

from oxidative damage, but can also react with chemical toxins such as carbon tetrachloride and benzene (Vichnevetskaia et al. 1999).

If an increase in pollock intake results in a loss of vitamin E, and an increased chance of oxidative damage as a result, then the decline of the Steller sea lions could be related to an increase in illnesses related to oxidative damage and a higher mortality rate as a result. Studies on rats have shown that a deficiency in vitamin E can cause neuropathy as evidenced by increased levels of oxidation in all neural tissues (MacEvilly et al. 1996).

The loss in serum vitamin E caused by the pollock diet can be due to a lowered dietary vitamin E intake from pollock than herring. It could also be due to pollock having a higher polyunsaturated fat content than herring, which is higher in saturated fat than pollock. PUFA is very susceptible to lipid oxidation due to the double bonds in the chemical structure of the fatty acids. The dietary and body stores of vitamin E in the captive sea lions may have been used up to combat this oxidation. Subsequently, dietary vitamin E may not have been enough to replenish the vitamin E pool in the body. A high level of dietary vitamin E has been shown to suppress both enzymatic and non-enzymatic lipid oxidation in rats (Sodergren et al. 2001), so it is an important dietary component. Chronic dietary vitamin E deficiency in rats resulted in undetectable levels of vitamin E in serum and liver after one year and minimal amounts in the muscle and nervous system (MacEvilly et al. 1996).

The hypothesis that a lowered serum vitamin E concentration may lead to oxidative stress was supported by the in vitro forced peroxidation experiment with the red blood cells of the three sea lions. A drop in plasma levels of vitamin E coincided with a reduced ability to protect against oxidative challenges in all three sea lions (Figs. 3.2, 3.3, and 3.4). Another study found that rats fed a diet supplemented with menhaden oil had increased levels of

omega-3 fatty acids in heart tissue, which in turn, caused the heart tissue to be more susceptible to oxidation than in rats fed corn oil or lard (O'Farrell et al. 1997). This effect occurred regardless of what oil supplement the rats were given (menhaden oil, corn oil, or lard), although the greatest levels of oxidation occurred from a combination of low dietary vitamin E and increased dietary intake of menhaden oil.

Deficiencies in vitamin E can also have effects on the fertility and reproduction of animals (Azzi et al. 2000). One study involving bovine embryos created in vitro found that vitamin E allowed more embryos to develop into blastocysts and that embryos cultured with vitamin E were larger in surface area than controls (Olson et al. 2000). Cows supplemented with vitamin E were also found to need fewer numbers of days and inseminations for conception to occur (Baldi et al. 2000). Such parameters are difficult to assess in wild populations of the Steller sea lion, but there is data that suggests lactating females had reduced pregnancy rates during the population decline (Pitcher et al. 1998).

There is some evidence of stress among Steller sea lions from declining populations. Plasma haptoglobin levels, which are an indicator of disease or sub-lethal damage, were elevated in sea lions from declining populations when compared to sea lions from stable populations (Zenteno-Savin et al. 1997). Haptoglobin can increase in response to inflammation, infections, trauma, myocardial infarction, rheumatoid arthritis, leukemia, and tuberculosis and other conditions (Zenteno-Savin et al. 1997). A number of these conditions can originate from oxidative stress but also the lower levels of vitamin E in sea lions fed pollock. Vitamin E also has a role in the prevention of disease and is found in higher concentration in immune cells than in other cells of the body, suggesting that immune cell membranes are at greater risk of oxidation (Wang et al. 2000). Studies have shown that

vitamin E supplementation improves cell-mediated immunity and oxidative stress in humans (Azzi et al. 2000; Lee et al. 2000). The lowered plasma vitamin E levels in sea lions fed pollock may result in an increased incidence of disease and infection. From my results with the captive sea lions, it is apparent that the red blood cells of the animals did indeed become more susceptible to oxidative damage, both from increased dietary PUFA and low serum vitamin E.

Future Directions

Although I obtained some promising results in my study in linking nutritional status with the decline of the Steller sea lion population, more work needs to be done to find the specifics of why pollock is detrimental to the sea lions' health. Of particular interest was the lowered vitamin E status in the animals fed the pollock diet. However, a larger sample size would increase the reliability of the results. As well, it would be helpful to analyze the amount of vitamin E in the pollock itself so that we may know if the problem lies in the content of vitamin E, or its bioavailability from the pollock. Perhaps post-mortem analysis on sea lions carcasses found in the areas of sea lion decline would also give clues as to whether the sea lion in declining populations are under oxidative stress. If cancers, neuropathy, cardiovascular disease or other types of diseases caused by oxidation are apparent, then we could better show a causative link between the pollock diet, oxidative stress, and the decrease in Steller sea lions numbers in Alaska.

Summary

Declining populations of Steller sea lions (*Eumetopias jubatus*) in Alaska have been consuming higher proportions of low-energy prey than they did in the past. My study compared the nutritional status of three captive Steller sea lions fed pollock (the current primary prey in the wild) and herring (the historic dominant prey). Compared to herring, all three animals increased the amount of pollock they ate to compensate for its lower energy density, and either lost weight or failed to gain weight at the same rate. No significant changes were detected in the nutritional status of the sea lions in terms of common hematology parameters such as plasma glucose, blood-urea nitrogen, and hematocrit. However, there was a decrease in plasma vitamin E concentration as well as a decrease in plasma total cholesterol for all three animals. When the red blood cells from sea lions fed pollock or herring were challenged with hydrogen peroxide, they exhibited increased susceptibility to oxidation when the sea lions were on the pollock diet. Thus the captive Steller sea lions were nutritionally stressed while on the pollock diet.

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Appendix I : Summary of Proximate Analysis on Pollock[1]

Month	Moisture %	Ash %	Lipid %	Protein %	Energy kcal/g	n
January	77.44 ± 0.180	12.23 ± 0.341	14.02 ± 0.450	68.54 ± 0.481	4.98 ± 0.033	83
February	73.28 ± 0.225	11.42 ± 0.450	17.44 ± 0.400	63.10 ± 0.883	5.23 ± 0.028	59
March	76.67 ± 0.201	11.68 ± 0.405	17.04 ± 0.677	62.48 ± 1.077	5.16 ± 0.037	61
July	76.33 ± 0.242	11.56 ± 0.424	21.81 ± 0.735	61.03 ± 0.657	5.34 ± 0.093	60
August	75.45 ± 0.221	9.65 ± 0.421	22.10 ± 0.624	63.31 ± 0.665	5.28 ± 0.051	61
September	75.49 ± 0.258	12.25 ± 0.523	21.43 ± 0.761	60.33 ± 0.881	5.23 ± 0.050	63
Oct-98	73.66 ± 0.261	10.11 ± 0.291	23.06 ± 0.674	59.49 ± 0.713	5.55 ± 0.036	76
Oct-99	73.79 ± 0.298	10.42 ± 0.362	20.44 ± 0.689	63.02 ± 0.629	5.43 ± 0.063	57
November	74.58 ± 0.173	10.52 ± 0.436	19.54 ± 0.656	61.40 ± 0.871	5.26 ± 0.078	60
$F_{8,571}$	36.597	5.687	24.516	14.176	10.557	
p	<0.001	<0.001	<0.001	<0.001	<0.001	

1. values are mean ± SEM

Appendix II: Difference in Proximate Analysis Values between sexes in pollock[1]

	Moisture %	Ash %	Lipid %	Protein %	Energy (kcal/g)	n
January						
Males	77.62 ± 0.237	12.20 ± 0.425	13.62 ± 0.625	68.48 ± 0.762	5.03 ± 0.040	46
Females	77.21 ± 0.282	12.23 ± 0.574	14.57 ± 0.662	68.72 ± 0.532	4.92 ± 0.055	36
February						
Males	76.56 ± 0.303	12.46 ± 0.735	17.11 ± 0.596	63.27 ± 1.385	5.21 ± 0.048	30
Females	75.98 ± 0.328	10.35 ± 0.440	17.78 ± 0.535	62.93 ± 1.108	5.25 ± 0.030	29
March						
Males	76.65 ± 0.239	12.06 ± 0.473	17.49 ± 1.088	61.32 ± 1.556	5.11 ± 0.052	33
Females	76.69 ± 0.341	11.23 ± 0.683	16.51 ± 0.739	63.84 ± 1.451	5.22 ± 0.049	28
July						
Males	76.47 ± 0.413	12.31 ± 0.728	22.16 ± 0.828	60.65 ± 0.794	5.27 ± 0.100	30
Females	76.19 ± 0.259	10.81 ± 0.403	21.46 ± 1.226	61.42 ± 1.055	5.41 ± 0.156	30
Aug						
Males	75.28 ± 0.361	9.80 ± 0.553	22.11 ± 0.786	63.18 ± 0.862	5.37 ± 0.070	30
Females	75.64 ± 0.269	9.64 ± 0.643	22.13 ± 1.008	63.27 ± 1.043	5.20 ± 0.045	30
September						
Males	75.35 ± 0.410	12.35 ± 0.808	20.99 ± 0.883	61.62 ± 0.938	5.22 ± 0.072	33
Females	75.65 ± 0.306	12.13 ± 0.661	21.91 ± 1.279	58.91 ± 1.510	5.24 ± 0.069	30
Oct-98						
Males	72.27 ± 0.250	10.26 ± 0.383	22.59 ± 0.899	60.13 ± 0.960	5.53 ± 0.044	30
Females	72.79 ± 0.350	9.73 ± 0.416	23.99 ± 1.031	58.26 ± 1.044	5.55 ± 0.065	44
Oct-99						
Males	73.86 ± 0.476	10.45 ± 0.555	21.48 ± 1.055	61.64 ± 0.833	5.54 ± 0.121	26
Females	73.7 ± 0.382	10.39 ± 0.486	19.58 ± 0.892	64.18 ± 0.880	5.35 ± 0.054	31
November						
Males	74.26 ± 0.256	11.43 ± 0.685	19.84 ± 0.810	60.95 ± 0.989	5.29 ± 0.096	30
Females	74.90 ± 0.224	9.61 ± 0.498	19.25 ± 1.043	61.84 ± 1.448	5.23 ± 0.125	30
Sex $F_{1,558}$	1.309	8.652	0.003	0.212	0.387	
p	0.253	0.003	0.957	0.646	0.534	
Month $F_{8,558}$	36.944	5.610	24.139	14.509	10.197	
p	<0.001	<0.001	<0.001	<0.001	<0.001	
Sex-Month $F_{8,558}$	2.059	0.992	0.723	1.285	1.08	
p	0.038	0.441	0.671	0.249	0.375	

1. values are mean ± SEM

Appendix III : Summary of Morphological Data Collected from Ground Pollock and Herring[1]

	Weight (g)	Length (cm)	Girth (cm)	Gonad weight (g)	GSI[2]	n
January	524.3 ± 11.10	38.6 ± 0.27	18.3 ± 0.20	25.2 ± 2.63	4.51 ± 0.377	83
Males	5.23.2 ± 12.06	38.6 ± 0.28	18.4 ± 0.17	18.7 ± 1.37	3.51 ± 0.234	46
Females	530.6 ± 20.01	38.8 ± 0.49	18.1 ± 0.41	34.3 ± 5.5	5.90 ± 0.754	36
February	670.0 ± 14.87	41.5 ± 0.30	20.2 ± 0.18	44.5 ± 4.48	6.37 ± 0.569	59
Males	643.9 ± 15.28	40.9 ± 0.42	19.9 ± 0.21	24.6 ± 2.76	3.67 ± 0.354	30
Females	697.1 ± 25.12	42.1 ± 0.39	20.4 ± 0.31	64.8 ± 6.87	9.16 ± 0.826	29
March	612.9 ± 13.24	40.7 ± 0.29	19.0 ± 0.15	38.0 ± 2.98	6.02 ± 0.403	61
Males	597.4 ± 14.05	40.5 ± 0.33	18.9 ± 0.18	25.7 ± 1.55	4.31 ± 0.244	33
Females	631.1 ± 23.46	41.0 ± 0.50	19.2 ± 0.25	52.5 ± 5.03	8.04 ± 0.653	28
July	527.6 ± 22.20	39.5 ± 0.61	18.4 ± 0.23	6.2 ± 0.83	0.96 ± 0.119	60
Males	490.7 ± 26.96	38.6 ± 0.87	18.2 ± 0.32	3.1 ± 0.64	0.46 ± 0.102	30
Females	564.5 ± 34.42	40.3 ± 0.85	18.7 ± 0.32	8.5 ± 1.20	1.46 ± 0.173	30
Aug	266.4 ± 6.72	31.9 ± 0.30	15.0 ± 0.17	2.1 ± 0.18	0.63 ± 0.059	61
Males	272.6 ± 7.90	32.5 ± 0.38	15.1 ± 0.20	2.6 ± 0.31	0.65 ± 0.104	30
Females	261.8 ± 11.08	31.5 ± 0.46	14.9 ± 0.29	1.8 ± 0.20	0.64 ± 0.055	30
September	678.6 ± 23.10	42.2 ± 0.48	19.7 ± 0.24	16.6 ± 2.30	2.34 ± 0.301	63
Males	674.2 ± 29.75	42.1 ± 0.55	19.7 ± 0.33	13.7 ± 2.00	1.94 ± 0.213	33
Females	683.5 ± 36.33	42.3 ± 0.82	19.7 ± 0.34	19.8 ± 4.27	2.78 ± 0.582	30
Oct-98	658.9 ± 19.11	40.8 ± 0.40	19.6 ± 0.20	13.7 ± 1.41	1.94 ± 0.195	76
Males	643.5 ± 19.75	40.4 ± 0.42	19.5 ± 0.24	12.9 ± 1.58	1.93 ± 0.218	30
Females	696.0 ± 37.10	41.6 ± 0.75	19.9 ± 0.34	14.7 ± 2.63	2.08 ± 0.370	44
Oct-99	917.2 ± 23.32	47.5 ± 0.55	22.5 ± 0.25	22.7 ± 3.11	2.42 ± 0.357	57
Males	876.4 ± 30.92	46.5 ± 1.01	22.0 ± 0.33	17.9 ± 2.30	1.91 ± 0.247	26
Females	951.5 ± 33.36	48.4 ± 0.55	23.0 ± 0.34	26.7 ± 5.24	2.85 ± 0.318	31
November	709.0 ± 24.37	44.1 ± 0.54	21.2 ± 0.31	35.3 ± 5.57	4.67 ± 0.772	60
Males	728.6 ± 37.77	43.9 ± 0.81	21.2 ± 0.41	29.9 ± 4.96	3.77 ± 0.520	30
Females	689.5 ± 31.05	44.3 ± 0.73	21.1 ± 0.46	40.7 ± 10.22	5.56 ± 1.449	30
Herring	43.2 ± 1.19	14.8 ± 0.13	8.0 ± 0.09	2.3 ± 0.23	5.23 ± 0.487	68
Males	42.4 ± 1.27	14.7 ± 0.13	8.0 ± 0.09	3.6 ± 0.28	8.36 ± 0.521	33
Females	43.9 ± 2.04	14.8 ± 0.23	8.0 ± 0.17	1.3 ± 0.23	2.49 ± 0.385	32

1. values are mean ± SEM 2.Gonadosomatic index = gonad weight / body weight x100