CHANGES IN BLOOD PARAMETERS, MUSCLE MYOGLOBIN AND MUSCLE LACTATE DEHYDROGENASE OF THE COMMON MURRE (*Uria aalge*) DURING MATURATION

by

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Blood oxygen carrying capacity, myoglobin levels and LDH isozyme compositions in the heart, gastrocnemius and pectoralis muscles were determined in Common Murre adults and during maturation of the chick at sea. Oxygen stores in the chick (hemoglobin, hematocrit, muscle myoglobin) increased significantly with growth. High levels of the aerobic isozyme, LDH 1, were maintained throughout maturation in the heart. All five LDH isozymes were maintained at similar levels in the gastrocnemius muscle. The pectoralis showed an increase in LDH 1, 2, 3, and 4, yet retained relatively high levels of LDH 5 throughout maturation. Upon leaving the nesting colony, metabolic capacities in the heart and gastrocnemius of the chick are similar to those of adults. The chick pectoralis tissue,

however, gains aerobic capacities with maturation which is concomitant with the needed capacity for aerial and aquatic flight upon fledging.

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CHAPTER I

INTRODUCTION

Diving marine birds and mammals utilize several physiological and biochemical strategies that allow them to preserve enantiostasis while submerged (Mangum and Towle, 1977). Given the lack of available environmental oxygen when diving, one strategy to maintain metabolic function during prolonged submergence is to increase the body's oxygen storage capacity (Weber, et. al., 1974; Baldwin, et. al., 1984). In the blood, this is accomplished through an increase in hemoglobin concentration, which heightens the oxygen carrying capacity (Butler and Jones, 1982). Enhancing muscle myoglobin concentration also serves as a valuable tissue oxygen store and facilitates intracellular oxygen transfer within the muscle as well as from the blood (Butler and Jones, 1982; Hochachka and Guppy, 1987).

Even with these mechanisms for storing oxygen, the fact remains that during prolonged diving, available oxygen can become severely limited. Forced submergence experiments on restrained animals led to characterization of the "diving response" - a set of physiological reflexes employed by the animal to forestall hypoxia and maximize dive duration. Of

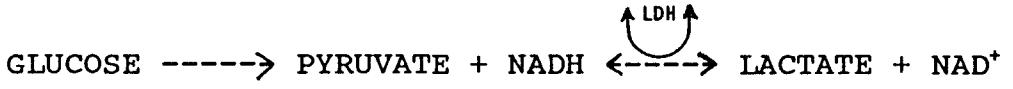
these diving reflexes, bradycardia and peripheral vasoconstriction were thought paramount to conserving oxygen (Sholander,1940; Butler and Jones, 1982). Bradycardia and subsequent metabolic depression reduce overall oxygen requirements (Hochachka and Somero, 1984). Peripheral vasoconstriction preferentially supplies an oxygen rich blood reserve to the central organs (heart, lungs, brain) but forces reduced circulation to peripheral organs, such as skeletal muscle, which then must metabolize anaerobically via glycolysis (Butler and Jones, 1982; Hochachka and Somero, 1984). When the animal is utilizing "dive response" strategies, lactate, the end product of glycolysis, can accumulate in unperfused muscle tissue leading to metabolic acidosis (Butler and Jones, 1982; Hochachka and Somero, 1984). Consequently, animals used in forced submergence experiments showed large post dive lactic acid concentrations in the blood as complete circulation was restored (Castellini, et. al., 1988).

Conserving oxygen for the sensitive, aerobic-dependent tissues while other tissues relied on anaerobic metabolism was thought critical to successful diving in marine animals. However, recent studies on unrestrained, freely diving birds and mammals have altered views concerning the cardiovascular and metabolic adjustments that occur during diving (Kanwisher, et. al., 1980; Butler, 1982). Contrary to the classical "dive response", the majority of voluntary dives

are aerobic with minimal bradycardia and peripheral vasoconstriction (Butler and Jones, 1982; Butler, 1988). Moreover, when freely diving, a marine bird or mammal is physically active, which is likely to initiate a physiological response similar to sustained exercise, such as an increase in heart rate and cardiac output, along with an increase in metabolism and peripheral vasodilation (Butler, 1988). Thus, given the metabolic paradox of increased demand yet limited supply of oxygen during diving, an actively diving animal must regulate the use of its oxygen supply during exercise. This ensures that tissues with only aerobic capacity are constantly supplied with stored oxygen. Conversely, tissues with anaerobic alternatives to aerobic metabolism may employ those alternatives as oxygen stores are depleted. For example, sustained submaximal skeletal muscle work has high energy demands and requires oxygen and metabolic substrates to be supplied by unrestricted circulation (Hochachka and Guppy, 1987; Hoppeler and Billeter, 1991). However, peak skeletal muscle work is usually anaerobic and supported by intramuscular glycolytic substrates (Hoppeler and Billeter, 1991). Since both of these types of muscle activities are likely to occur when diving, metabolic plasticity would seem to be the optimal strategy for a diving animal.

The ability to adjust tissue requirements and enzymatic capacities is one of the methods thought to allow maximum

exercise performance while diving under limited oxygen conditions. Under these conditions of oxygen deprivation, the ability of the tissues to generate ATP and maintain a metabolic redox balance depends on the activity of gylcolytic enzymes (Everse and Kaplan, 1975). The enzyme, lactate dehydrogenase (LDH), catalyzes the final step of glycolysis in the reduction of pyruvate to lactate:



Thus, LDH is an integral part of this energy production in anaerobic metabolism in both forced and voluntary dives. Lactate dehydrogenase exists as a tetrameric molecule of two subunit types, H and M, that can combine to form five isozymes: H_4 (LDH 1), H_3M_1 (LDH 2), H_2M_2 (LDH 3), H_1M_3 (LDH 4), and M_4 (LDH 5) (Dawson, et. al., 1964). These isozymes differ in substrate specificities, enzyme kinetics, substrate concentration optima, and amino acid composition (Dawson, et. al., 1964; Everse and Kaplan, 1975). Studies of LDH enzyme kinetics have led to the theory that H type LDH is the predominant isozyme in aerobic metabolizing tissues and M type LDH is the main isozyme in anaerobic tissues (Vesell, 1968; Everse and Kaplan, 1975). This hypothesis has tempted researchers to correlate the levels of muscle tissue M-LDH with the diving abilities of marine

vertebrates, particularly seabirds and marine mammals.

The importance of physiological diving parameters including increased oxygen storage by increased hemoglobin and myoglobin concentrations, and anaerobic abilities as represented by LDH isozymes, may best be illustrated by examining the changes in those parameters in an organism that exhibits increasing diving ability with maturation. The Common Murre (Uria aalge) is an ideal subject for a study of this type. The Common Murre, a member of the family Alcidae, spends most of its life at sea, as far as 60km from shore, except during its breeding season. The murre is a proficient diving seabird and is highly specialized to pursue prey underwater using wing-propelled "flight" (Tuck, 1960; Spring, 1971). Common Murres routinely dive to depths of 180m and can remain submerged for up to four minutes (Piatt and Nettleship, 1985; Varoujean, unpubl. data). These data, based on incidences of murres caught in gill nets at varying depths to 200m, and dive times of adult murres foraging for their chicks, suggest that murres are not stressed at these depths, and may dive even deeper (Piatt and Nettleship, 1985). Furthermore, murres are capable of extended aerial flights of tens of kilometers and can sustain flight speeds of approximately 70 kilometers per hour (Pennycuick, 1987a,b).

The breeding biology of the Common Murre is of interest with respect to its ontogeny and diving abilities. Common

Murres nest in large colonies on steep rocky island cliffs. Incubation and feeding of the single chick on the colony site is shared by both parents (Birkhead, 1976). After 18-25 days the chick jumps off the colony and disperses to sea accompanied by the male parent (Tuck, 1960; Birkhead, 1977; Varoujean, et. al., 1979; Scott, 1990). Upon leaving the colony, the chick is unable to fly or dive effectively and must surface swim to keep up with the male parent. The parent must dive frequently to obtain enough food for its own physiological maintenance needs and to maintain a positive growth rate for its chick (Sanford and Harris, 1967; Varoujean, unpubl. data). Parental care at sea usually lasts for 45-60 days, after which time the chick is fully developed (Varoujean, et. al., 1979). Toward the end of this period, the chick eventually attempts to follow its parent on foraging dives. At the end of this parental care period, the chick, now capable of aerial flight, is abandoned by the parent. The fact that the Common Murre chick makes an abrupt transition from sedentary colony life to constantly paddling surface swimmer to diver and flier in approximately two months, raises a fundamental question: What physiological and biochemical changes are taking place during maturation that enhance the murre's exercising and diving abilities?

A Common Murre diet composition study, in which murres were collected at sea during the murre's nesting season,

allowed analysis of some physiological parameters associated with diving capacities of murres at varying stages of maturity. Focus was on three muscle groups: (1) the heart, a muscle that relies exclusively on aerobic metabolism; (2) the gastrocnemius, a leg muscle the murre uses predominantly for sustained exercise in the form of surface swimming; and (3) the pectoralis, a breast muscle likely to require the capacity for sustained aerobic exercise during aerial flight as well as anaerobic work under limited oxygen conditions, i.e., aquatic flight. Specifically, the following questions were addressed in this study: Do the blood parameters of hemoglobin concentration and hematocrit change with maturation in the Common Murre? Do the myoglobin concentrations in the heart, gastrocnemius and pectoralis muscles change with maturation? Do the lactate dehydrogenase activities and isozyme compositions in the heart, gastrocnemius and pectoralis muscles change with maturation? How do these changes relate to the adult Common Murre's diving abilities?

CHAPTER II

MATERIALS AND METHODS

Animal Collection

Tissue and blood samples used in this study were taken from Common Murres (Uria aalge) collected specifically for stomach content analysis to document the seabird's local abundance, distribution and feeding habits along the southern Oregon coast. Collection of murres at sea occurred within a five kilometer radius of the Coos Bay, Oregon harbor entrance, including lower Coos Bay waters. Sixty five Common Murres of varying ages were used in this study (Appendix A). Collection by boat began when chicks were first seen on the water accompanied by adults (3 July, 1987), and ended when fully grown chicks were seen in nonbreeding plumage, not accompanied by adults (7 October, 1987). Toward the end of the nesting season, a fully grown murre chick, in nonbreeding plumage, is easily distinguished from a similarly sized adult because the adult is either in breeding plumage or is in the process of molting. Tissue samples from four adults collected in January 1988 for other purposes were also used in this study. Each Common Murre

was killed with a 12 gauge shotgun and immediately retrieved. The body cavity was opened to expose the heart and approximately 3-5 milliliters (ml) of blood was obtained by ventricular puncture with an 18 gauge needle and 5ml syringe treated with ethylenediaminetetraacetic acid (EDTA) to prevent clotting. Blood was immediately injected into 3.5ml EDTA treated vacuum tubes, labelled and put on ice. The gut tract and contents were then removed for the diet analysis study, bagged, labelled and put on ice. As allowed by calmer seas, the entire pectoralis muscle, both legs (including bone), and the heart muscle were removed in the field, placed in whirlpacks, labelled and put on ice. In rougher seas (swells 6+ feet, winds 30+ knots), whirlpacks were filled with ice and placed inside body cavities, carcasses were then placed on ice, and tissues were dissected immediately upon returning to the lab, within one hour of collection. All tissues were weighed and then stored at -70°C until used. Common Murre carcasses were weighed, sexed, culmen length was measured, and age estimated (Appendix A). Age, in days from leaving the nesting colony, was calculated for each chick using the regression formula, culmen=18.7+0.38(age), (Varoujean, unpubl. data). For some analyses, birds were separated into five age groups: youngest chicks with nestling plumage head down under parental care (HD); young chicks under parental care (YP); older chicks under parental care (OP);

independent chicks no longer under parental care (IC); and adults (AD). Weights of dissected tissue samples and gut tract samples were added to their respective carcass weights to obtain a final, total body weight. Blood samples collected were analysed immediately for hemoglobin concentration and hematocrit.

Blood Parameters

Before analysis, blood samples were placed on a shaker and gently agitated until warmed to room temperature. Aliquots were taken up in untreated microcapillary tubes and spun for 3 minutes in an International IEC Micro-capillary centrifuge (model MB) to determine blood hematocrit. Height of plasma and height of packed red blood cells were measured, and this ratio was used to calculate the percent of red blood cells/blood volume.

Hemoglobin (Hb) concentration was measured spectrophotometrically via conversion to cyanmethemoglobin using a Sigma Diagnostics Total Hemoglobin Kit, procedure #525. After cyanmethemoglobin formation, the absorption of each sample was read at 540 nanometers using a Beckman DU70 spectrophotometer. Given that 1 millimole (mM) of heme has an extinction coefficient of 11, and 1 mole (M) heme corresponds to approximately 16,000 grams (g) protein per 100 ml blood (Kampen and Zijlstra, 1965), the concentration

of Hb was calculated using the formula: concentration= [(absorbance)(dilution factor)/11][1.6].

Tissue Preparation

Tissues were thawed partially for one hour at room temperature. A total of 0.2g wet weight of tissue for each muscle type (pectoralis, gastrocnemius, heart ventricle), was selected from several representative sites in each muscle sample. Tissue samples were immediately homogenized in 4.0ml of ice cold 0.1 molar (M) sodium phosphate buffer, pH 7.4, for 1.5 minutes using a Tekmar SDT-100EN Tissumizer. The homogenate was then centrifuged at 15,000g and 4°C for 30 minutes (in a Sorvall RC2-B refrigerated centrifuge). Supernatants were pipeted into vials and stored on ice until assayed and electrophoretically analysed.

Lactate Dehydrogenase

Enzyme Assay

Lacate dehydrogenase (LDH) activity was measured spectrophotometrically based on the change in molar absorbance of the co-substrate nicotinamide adenine dinucleotide (NADH) as it is oxidized into NAD^+ with the conversion of pyruvate into lactate (James, 1978). Enzyme

assays for each tissue supernatant were performed at 37°C, approximating the internal temperature of Common Murres (Davis and Guderly, 1987). Assay sets were carried out using a Beckman DU70 spectrophotometer with temperature controlled cuvette holder connected to a 37°C circulating water bath. Enzyme supernatant samples were diluted 1:2 with cold 0.1M sodium phosphate buffer (pH 7.4) and were held on ice to prevent protein denaturation before use. Prior to its assay, an enzyme sample was brought to room temperature to hasten its equilibration to 37°C in the assay reaction mixture. LDH activity was measured with five different pyruvate concentrations: 0.15mM, 0.3mM, 1.5mM, 3.0mM, 15.0mM. The assay buffer, 2.13mM NADH and the five pyruvate concentrations were each incubated at 37°C in a circulating water bath for 30 minutes prior to the assay. Each assay was carried out in a 4.0ml cuvette. The reaction mixture contained 0.1ml enzyme supernatant (LDH concentration approximately 2.0×10^{-11}M), 0.1ml NADH, 2.7ml buffer, and 0.1ml pyruvate for a 3.0ml total reaction mixture volume. Buffer, NADH, and enzyme supernatant were placed in the cuvette; pyruvate was then added and mixed to initiate the reaction. A decrease in absorbance at 340nm was immediately recorded for 30 seconds at 600 readings per minute. Given the molar extinction coefficient of 6.22 for NADH in a 1cm cell pathlength at 340nm (Dryer and Lata, 1989), the activity of LDH (per tissue sample, grams wet

weight) was calculated for each pyruvate concentration as concentration of pyruvate (substrate) converted to lactate (product) per unit time (mol/gm/sec). Michaelis-Menton constants (Km values) of each enzyme sample were estimated by Direct Linear plots (Eisenthal and Cornish-Bowden, 1974). The Km values represent the concentration of pyruvate at which the LDH catalyzed reaction reaches half its maximum velocity, and are a measurement of the LDH reaction rate.

Electrophoresis

All enzyme samples were analysed for LDH isozyme composition by resolving with 5.5% polyacrylamide gel electrophoresis (PAGE) (Dietz and Lubrano, 1967). Samples were electrophoresed at 35 milliamps (mA) for approximately 2.5 hours at room temperature. Visualization of LDH isozymes was accomplished by "staining" with an enzymatic activity reaction that is specific for LDH. This reaction involves NADH in an oxidation-reduction chain of electron transfers (Dryer and Lata, 1989). Staining reactants were 1.5mM NAD^+, 0.5M D,L lithium lactate, 1.0mM phenazine methosulfate and 5.0mM nitroblue tetrazolium (Sigma Chemical Co.). Staining procedures were as described by Markert and Masui (1969). The last molecule in the chain of electron transfers, nitroblue tetrazolium, is a dye which is visibly blue in the reduced state:

$$\text{LACTATE} + \text{NAD}^{+} \xrightarrow{\text{LDH}} \text{PYRUVATE} + \text{NADH} + \text{H}^{+}$$

$$\text{NADH} + \text{H}^{+} + \text{PMS}_{ox} \longrightarrow \text{NAD}^{+} + \text{PMS}_{red}$$

$$\text{PMS}_{red} + \text{NBT}_{ox} \longrightarrow \text{PMS}_{ox} + \text{NBT}_{red} \quad \text{(blue/purple)}$$

After staining, LDH gels were sandwiched between two sheets of cellophane membrane backing (BioRad, model 543) and air dried. After complete dehydration, gels were stored in the dark to minimize UV degradation of the resolved LDH isozyme bands.

The isozyme bands present in each sample were quantified with video image analysis, using the computer program, JAVA (Jandel Scientific). Each gel was illuminated on a white light table (Vari Quest 100, Fotodyne). Through this video image analysis system, gel images were translated from a CCD high resolution video camera (Pulnix TM-7CN with AF Micro Nikkon 100mm lens) into a set of digital values, by a TARGA+ frame grabber computer video adapter board (Truevision Inc.). The frame grabber renders the gel image as a set of discrete picture element cells (pixels). Pixel brightness is defined by a gray scale of intensity ranging from 0 (white) to 255 (black). The gray level, or darkness, of the pixel is thus a measure of its intensity and is stored as a numeric value. Using JAVA, each gel image was "captured" and stored on disk. The darkness (intensity) of each isozyme image reflected the amount of staining of the

band and hence relative quantity of isozyme present. For each isozyme band in each sample, intensity was measured and values were transformed into a percent scale for ease of graphical display.

Myoglobin

Myoglobin concentration was determined electrophoretically by sodium dodecyl sulphate (SDS) PAGE on 14% slab gels. This SDS method separates myoglobin from other proteins, including hemoglobin (Hofmann and Buchel, 1991). Sample preparation procedures were carried out as described by Laemmli (1970). Briefly, supernatant samples were combined with SDS incubation buffer and thiol reagent, dithiothreitol, and heated to 100°C for 1.5 minutes. This process dissociates and denatures multichain proteins by breaking hydrogen and reducing disulfide bonds, facilitating electrophoretic resolution of globular proteins. Samples were then electrophoresed at 100 volts for approximately 4 hours at room temperature, stained for 8-12 hours with Coomassie brilliant blue R250, then destained with 10% acetic acid (Fairbanks et. al., 1971). Gels were stored in 10% acetic acid until analysed. Myoglobin bands were quantified as described above for LDH isozyme bands, using video image analysis (JAVA).

Statistical Analysis

Regression analyses were performed on blood hemoglobin concentration and hematocrit, myoglobin intensities of each muscle group, and Km values of each muscle group. Regressions were done only on the chick data to assess any change in these physiological parameters during maturation. Myoglobin data for the pectoralis muscle were log transformed to eliminate skewness and allow use of analysis of variance (ANOVA). Adult data were included in one way analysis of variance (ANOVA) on the hemoglobin, hematocrit and myoglobin data to assess any differences among the age groups: HD, YP, OP, IC, AD. LDH activity and LDH isozyme data were also grouped by age; LDH activity data were analysed at increasing pyruvate concentrations via one way ANOVAs to assess any differences between age groups at each of these pyruvate concentrations. Regression analyses were performed on each isozyme in each muscle tissue to assess any compositional changes during maturation. In the heart muscle, all five isozymes did not always occur. Therefore, regression on heart muscle isozymes only included chicks in which the particular isozyme being analysed was present. A Chi-square (X^2) contingency table analysis was also used to examine LDH isozyme frequency (presence, absence) distributions in heart muscle.

CHAPTER III

RESULTS

Blood Parameters

Regression analysis showed a significant ($p<0.001$) increase in hemoglobin concentration ([Hb]) with age in Common Murre chicks (Figure 1), with sixty percent ($r^2=.60$) of the variation in [Hb] explained by age in a strong positive linear relationship ($y=.093x+8.52$). Regression of hematocrit (Hct) with age data (Figure 2) was also significant ($p< 0.001$), but showed a higher degree of variability in the increase of blood Hct with age ($r^2=.35$). When separated into age groups to include adults, one way ANOVAs indicated a significant difference among the age groups ($p<0.001$). There was a 1.5-fold increase in mean values of [Hb] and Hct (Table 1) from youngest chicks (HD) to adults (AD).

Myoglobin

Of the three muscle tissues examined by regression analysis, myoglobin (Mb) intensity in the pectoralis muscle

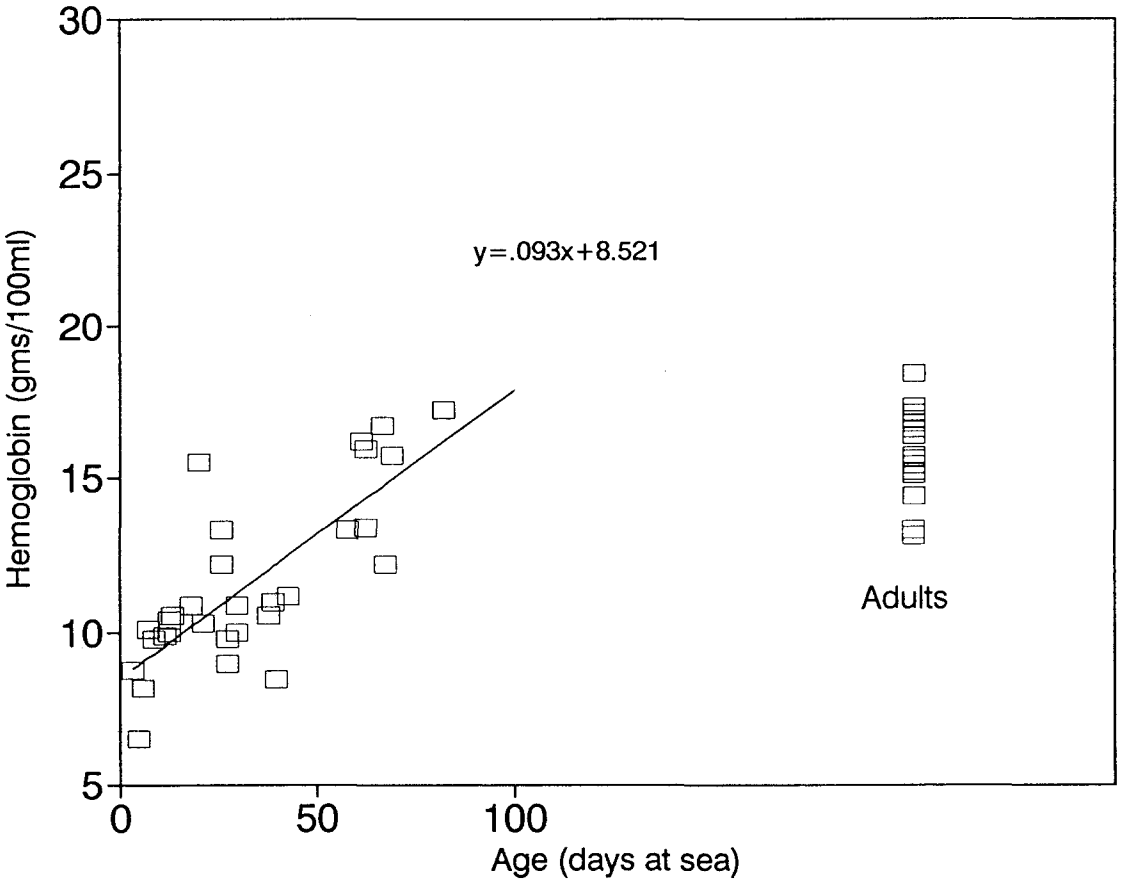


Figure 1. Hemoglobin concentration changes with age of Common Murre chicks. Regression (chicks only) r^2=.60 ($p< 0.001$). Adult values included for reference.

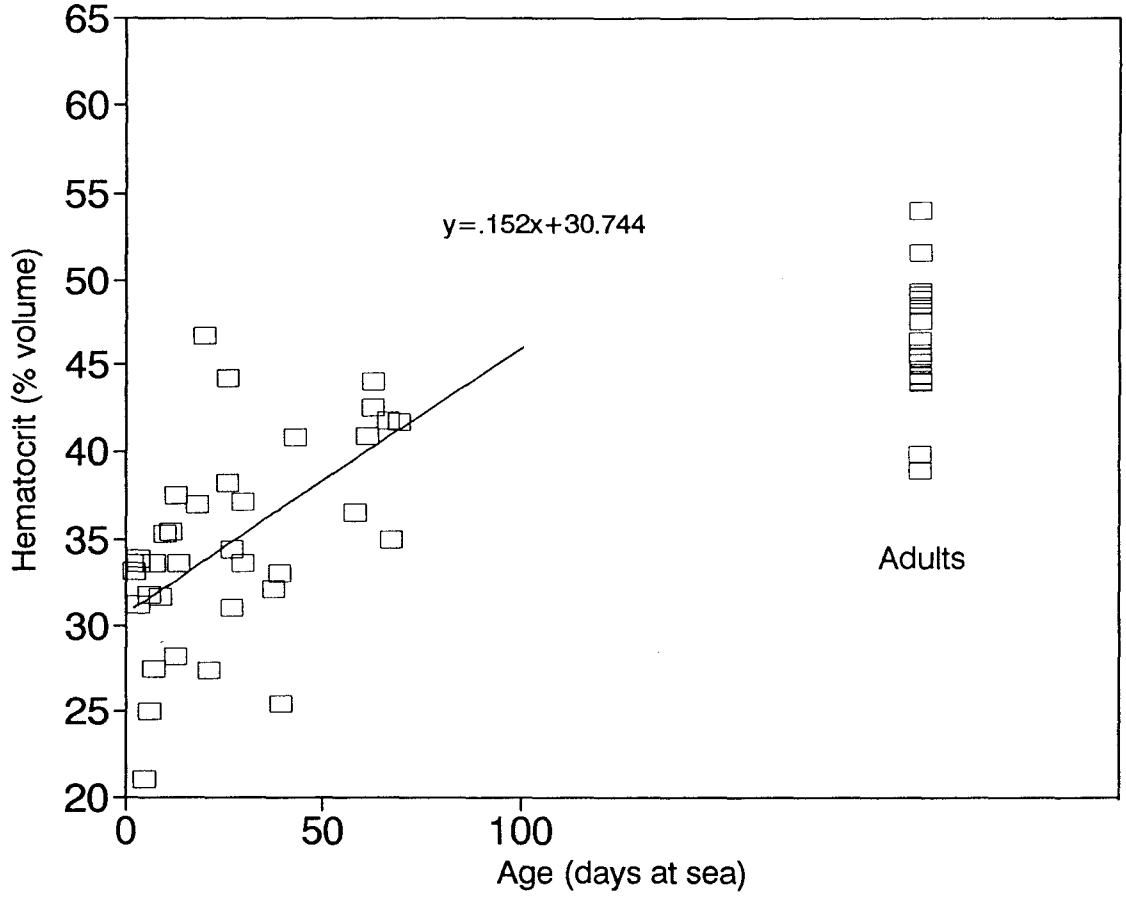


Figure 2. Hematocrit changes with age of Common Murre chicks. Regression (chicks only) r^2=.35 ($p< 0.001$). Adult values included for reference.

Table 1. Mean (± standard deviation) values of hemoglobin concentrations ([Hb]), hematocrit (Hct) and pectoralis, gastrocnemius and heart muscle myoglobin in five age groups* of the Common Murre.

Age Group	Sample Size(n)	[Hb] (g/100ml)	Hct (%)	Mb (intensity)	Muscle Tissue
HD	18	9.4 ±1.3[a]	31.7 ±4.5[b]	33.5 ±3.7	Pect.
				35.9 ±5.0	Gast.
				40.6 ±6.4	Heart
YP	9	11.3 ±2.0	36.3 ±6.1	33.1 ±3.2	Pect.
				34.3 ±4.4	Gast.
				37.1 ±3.5	Heart
OP	9	12.8 ±2.8	37.0 ±6.4	52.2 ±12.5	Pect.
				51.0 ±11.6	Gast.
				50.2 ±9.2	Heart
IC	7	15.4 ±1.7[c]	42.2 ±1.5[c]	58.6 ±12.3	Pect.
				57.0 ±11.8	Gast.
				50.9 ±11.2	Heart
AD	23	15.8 ±1.3[d]	46.3 ±3.7[d]	64.3 ±9.4	Pect.
				67.8 ±6.7	Gast.
				56.4 ±8.9	Heart

* Age groups represented: Youngest chicks with nestling plumage head down under parental care (HD); young chicks under parental care (YP); older chicks under parental care (OP); independent chicks no longer under parental care (IC); adults (AD). Superscripts denote sample sizes differing from column sample sizes: [a]n=9; [b]n=15; [c]n=3; [d]n=18.

(Figure 3) exhibited the strongest linear correlation with age (r^2=.66; p<0.001). Figure 4 shows that gastrocnemius Mb intensity also increases during maturation in chicks fairly predictably (r^2=.51; p<0.001). Although exhibiting a significantly (p=0.001) positive correlation with age, heart muscle Mb intensity (Figure 5) showed a fairly high degree of variability (r^2=.24) with maturation. Figure 6 depicts the mean Mb intensities of pectoralis, gastrocnemius and heart muscle in the five Common Murre age groups. For each muscle tissue ANOVA was significant (p<0.001), indicating differences in Mb intensity among age groups. Mean Mb values (Table 1) increased from youngest chick (HD) to adult (AD) almost 2-fold in pectoralis and gastrocnemius muscles, and 1.3-fold in the heart muscle.

Lactate Dehydrogenase

Enzyme Activitiy

ANOVA analysis of pectoralis muscle (Figure 7) LDH activity at the lowest two pyruvate concentrations, 0.15mM and 0.3mM, showed significant differences among age groups, p<0.05. Differences among age groups progressively diminished as pyruvate concentrations increased (1.5mM p=0.06; 3.0mM p=0.48; 15mM p=0.89). ANOVAs performed on the five pyruvate concentrations in the gastrocnemius muscle

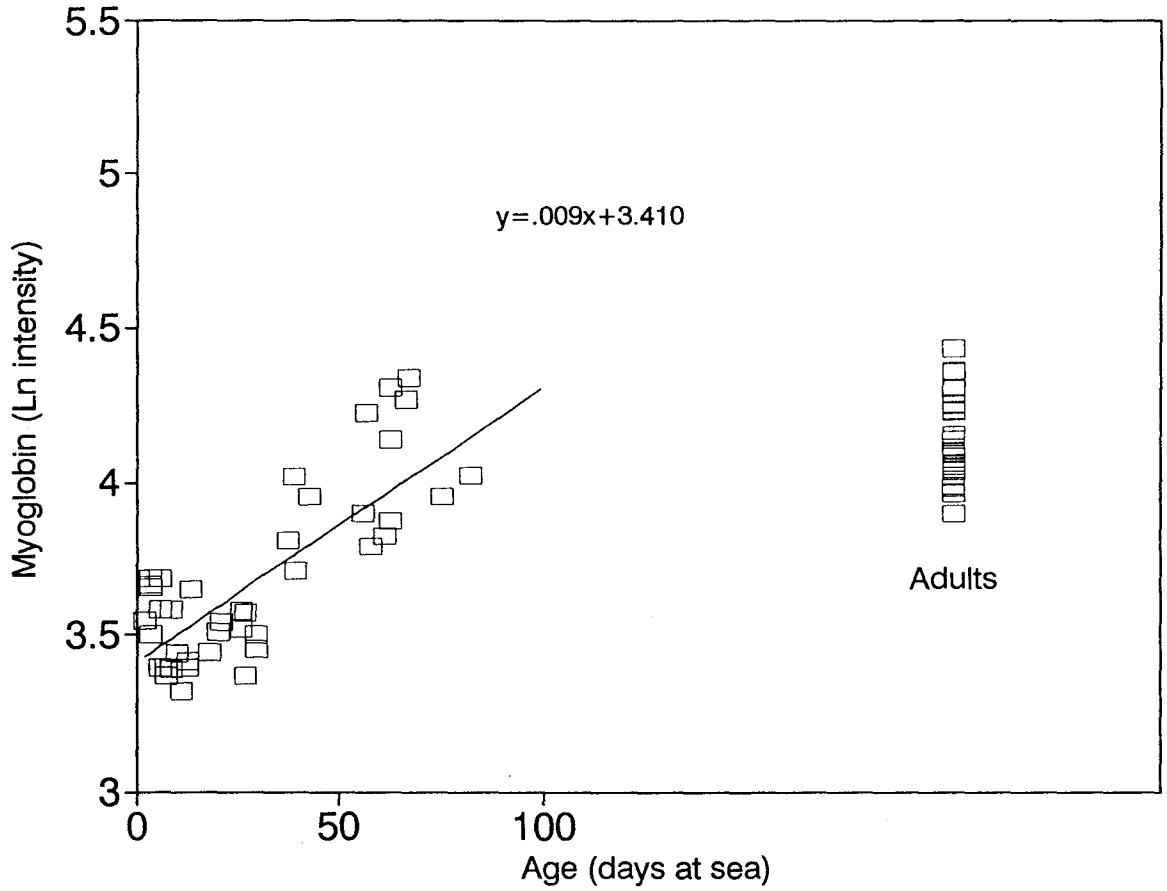


Figure 3. Pectoralis muscle myoglobin intensity (Ln transformed) changes with age of Common Murre chicks. Regression (chicks only) r^2=.66 ($p< 0.001$). Adult values included for reference.

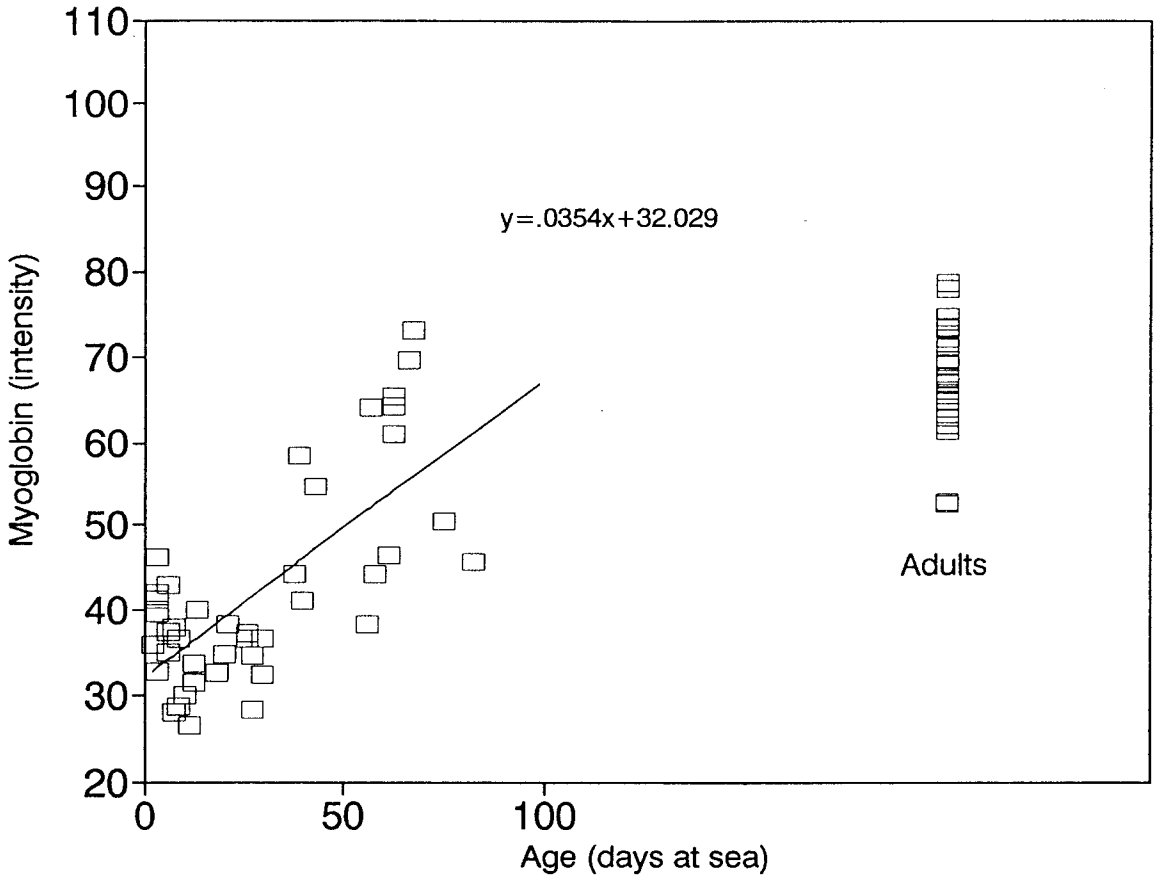


Figure 4. Gastrocnemius muscle myoglobin intensity changes with age of Common Murre chicks. Regression (chicks only) r^2=.51 ($p< 0.001$). Adult values included for reference.

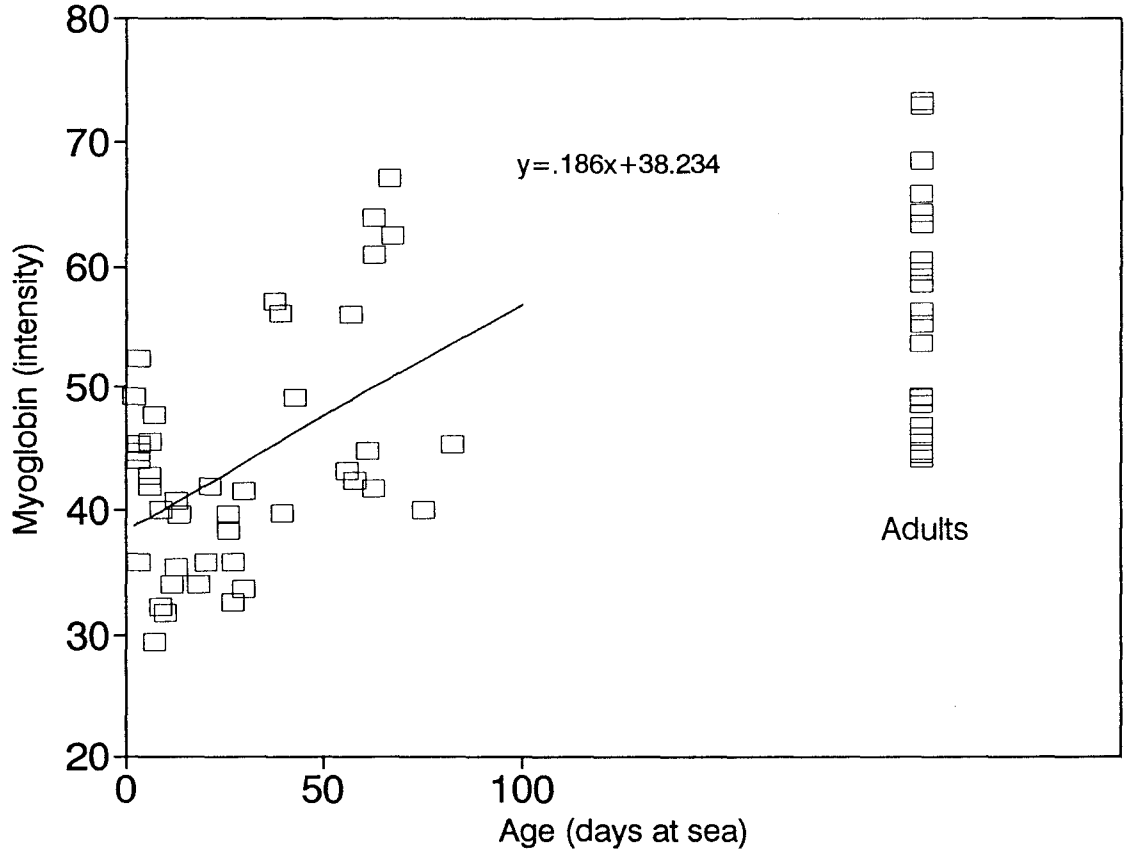


Figure 5. Heart muscle myoglobin intensity changes with age of Common Murre chicks. Regression (chicks only) r^2=.24 (p= 0.001). Adult values included for reference.

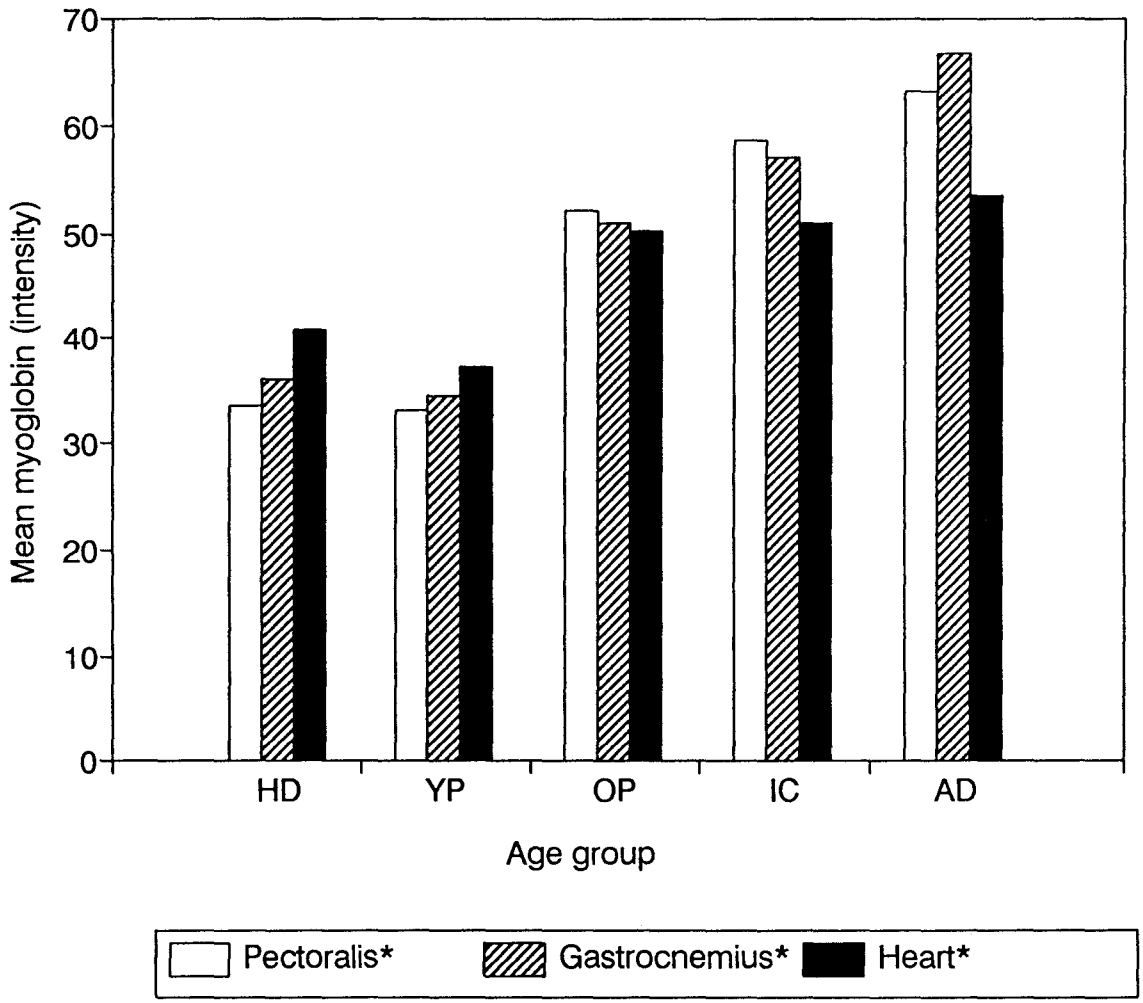


Figure 6. Mean myoglobin intensities of pectoralis, gastrocnemius, and heart muscle in five Common Murre age groups: Youngest chicks under parental care (HD); young chicks under parental care (YP); older chicks under parental care (OP); chicks no longer under parental care (IC); adults (AD).

*For muscle types differences in myoglobin intensity among age groups is significant at $p < 0.001$.

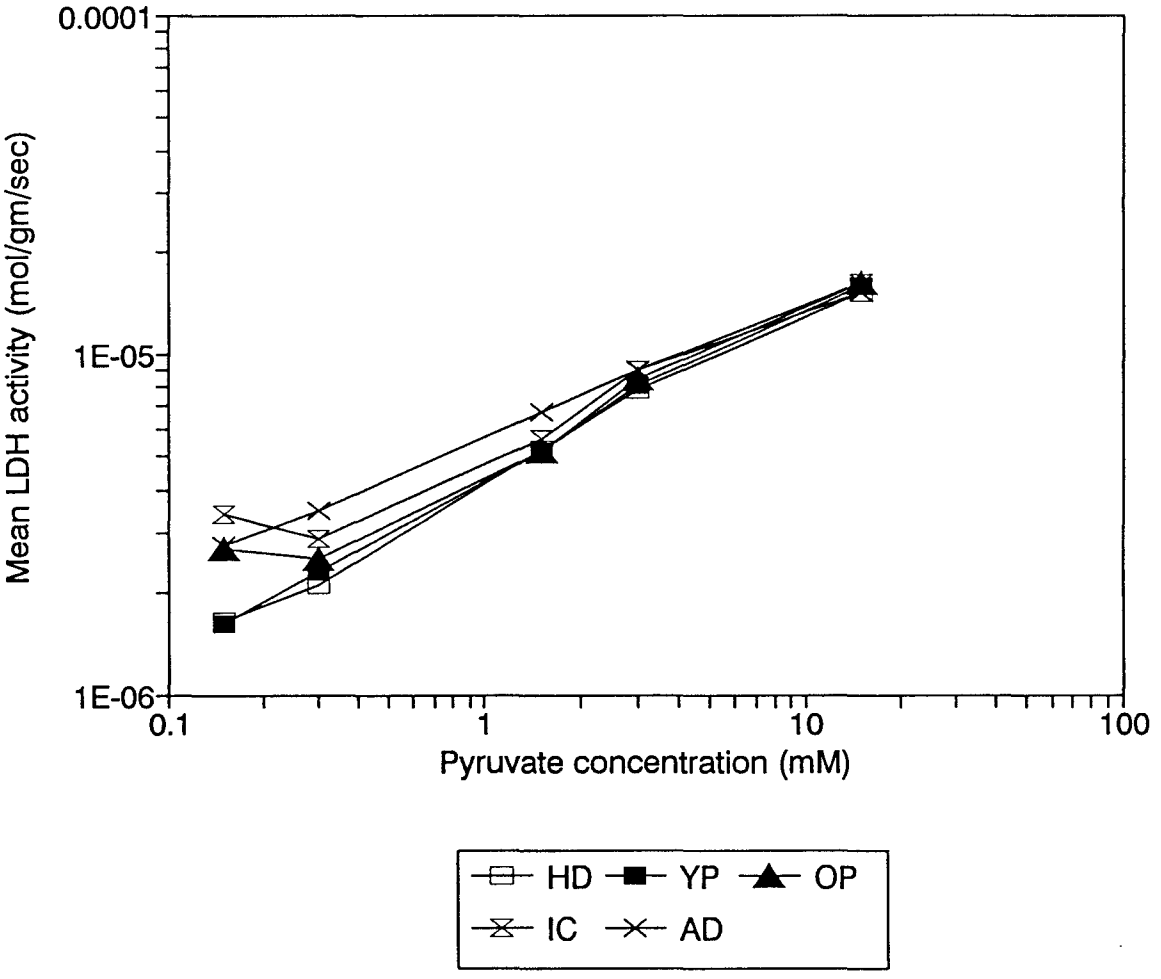


Figure 7. Pectoralis muscle LDH enzyme activity with increasing pyruvate concentration in five age groups of the Common Murre: Youngest chicks under parental care (HD); young chicks under parental care (YP); older chicks under parental care (OP); chicks no longer under parental care (IC); adults (AD). P values for differences between groups: $p<0.05$ for 0.15mM, 0.3mM; p not significant for 1.5mM, 3.0mM, 15.0mM.

(Figure 8) showed no significant differences among age groups. Figure 9 illustrates enzyme activities in the heart muscle. At pyruvate concentrations of 0.15mM, 0.3mM, 1.5mM, and 3.0mM ANOVA showed no significant differences in LDH activities among age groups; at 15.0mM, however, ANOVA did show difference among Common Murre age groups, $p=0.001$. Table 2 summarizes LDH activities at each pyruvate concentration in muscle tissues of the five age groups.

Regression analysis of Km values for LDH in the pectoralis (Figure 10) muscle showed no significant relationship with age ($y=^{-}.001x+.196$), $r^2=.05$, $p=0.17$; i.e., change or decrease in Km cannot be explained by maturation). Similarly, in the gastrocnemius muscle (Figure 11) no significant correlation between Km and age was seen ($r^2=.01$, $p=0.51$). Heart muscle Km regression analysis (Figure 12) also showed no relationship between Km value and age of chick ($r^2=.00$, $p=0.75$). Figure 13 illustrates mean Km values for each muscle tissue in each Common Murre age group. Gastrocnemius muscle showed a significant difference among age groups (ANOVA $p<0.05$), that difference apparently evident in the YP age group. Although mean Km values generally appear to decrease in each muscle tissue with age, high variability within age groups (Table 3), precludes any statistically significant differences among age groups.

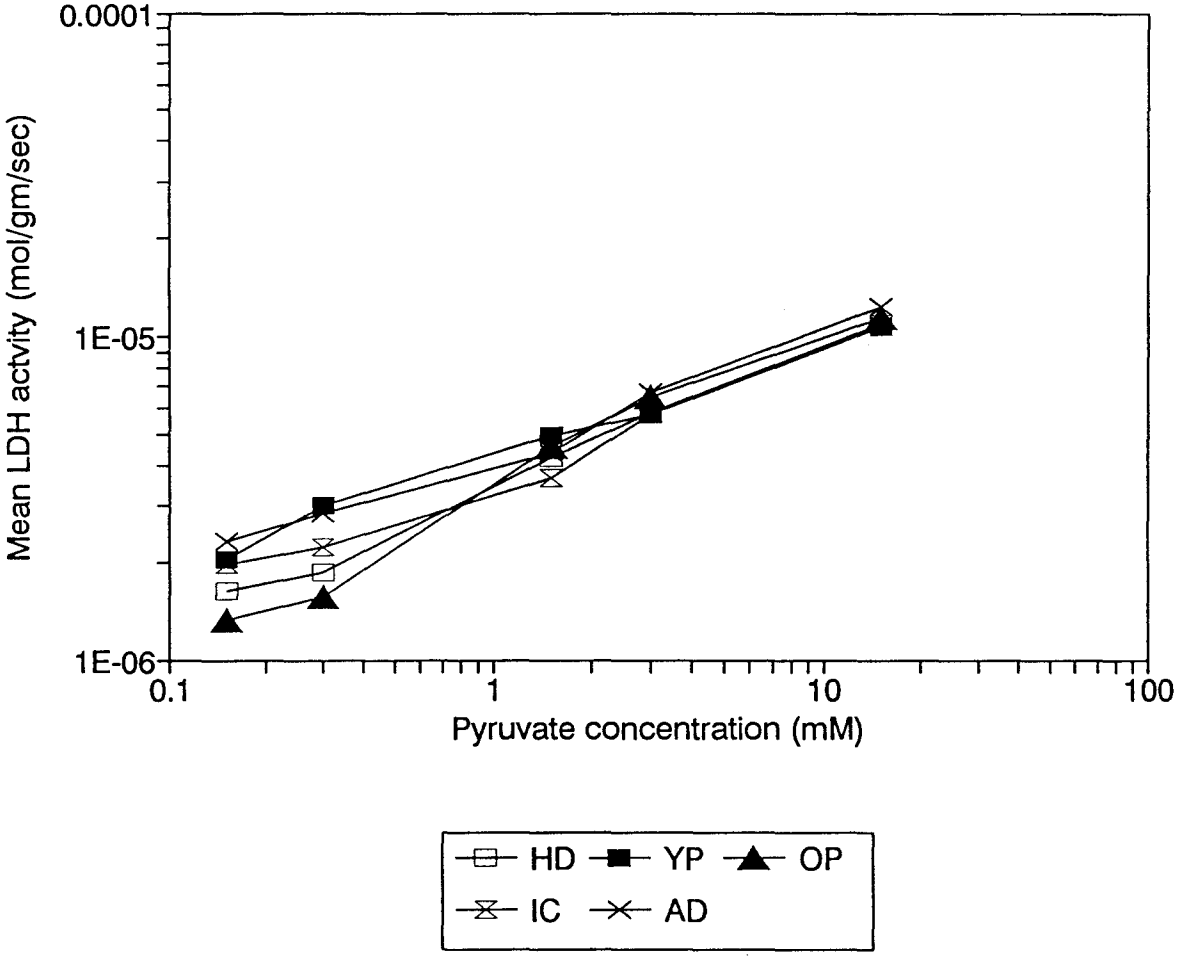


Figure 8. Gastrocnemius muscle LDH enzyme activity with increasing pyruvate concentration in five age groups of the Common Murre: Youngest chicks under parental care (HD); young chicks under parental care (YP); older chicks under parental care (OP); chicks no longer under parental care (IC); adults (AD). P values for differences between groups: p not significant for 0.15mM, 0.3mM, 1.5mM, 3.0mM, 15.0mM.

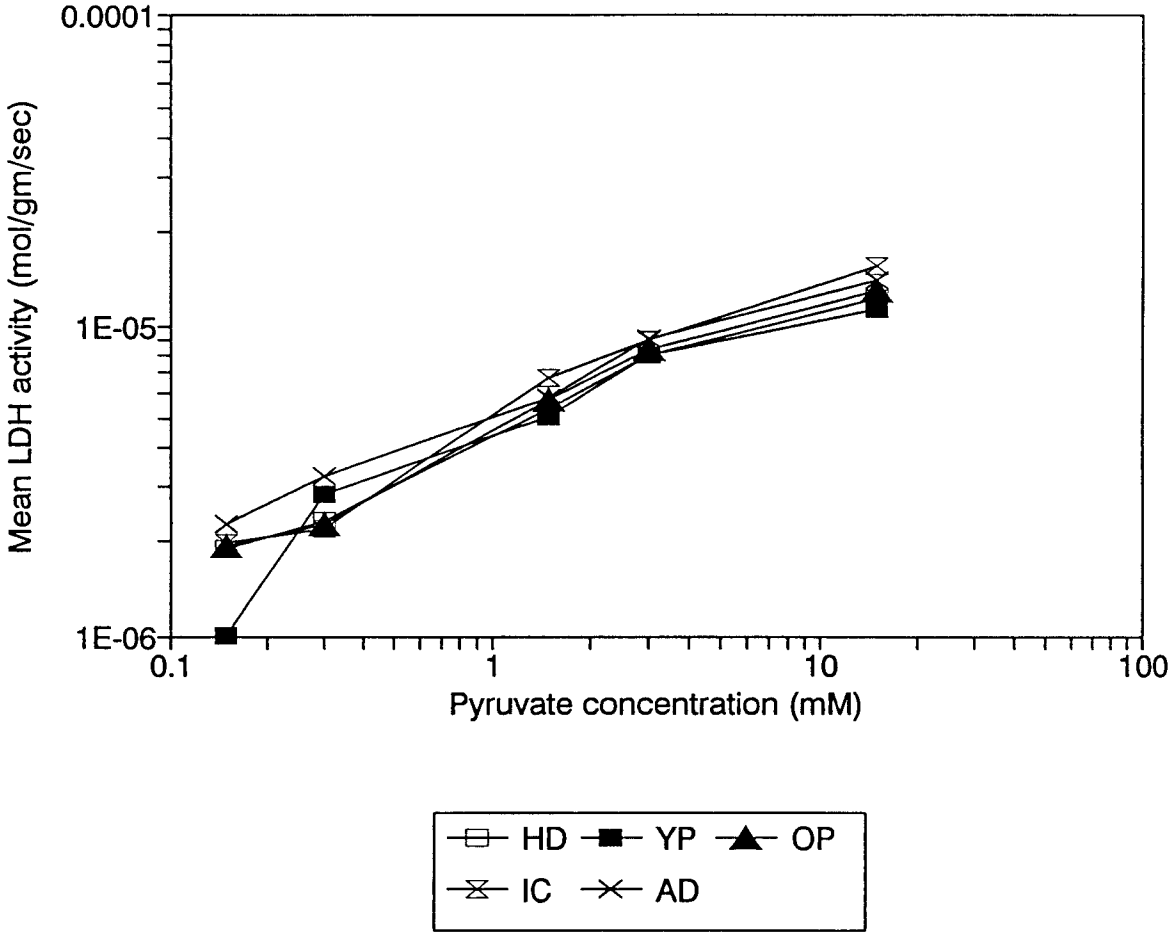


Figure 9. Heart muscle LDH enzyme activity with increasing pyruvate concentration in five age groups of the Common Murre: Youngest chicks under parental care (HD); young chicks under parental care (YP); older chicks under parental care (OP); chicks no longer under parental care (IC); adults (AD). P values for differences between groups: $p < 0.005$ for 15.0mM; P not significant for 0.15mM, 0.3mM, 1.5mM, 3.0mM.

Table 2. Mean (± standard deviation) LDH enzyme activities at five pyruvate concentrations in pectoralis, gastrocnemius and heart muscle in five age groups* of the Common Murre.

Muscle Tissue	Age Group	Sample Size (n)	Enzyme Activity (gm/mol/sec x 10^{-7}) 0.15mM	0.3mM	1.5mM	3.0mM	15.0mM
Pect.	HD	18	16.5±8.6	20.9±8.9	52.7±14.5	78.1±18.2	152.0±24.4
	YP	9	16.1±13.0	23.1±9.7	52.1±12.6	80.2±12.9	158.5±22.4
	OP	8	27.1±17.1	25.2±11.7	52.0±7.4	83.8±15.2	161.0±24.1
	IC	7	33.9±20.1	28.6±10.4	56.6±13.1	89.2±23.8	161.3±18.2
	AD	23	27.6±18.2	34.7±19.6	67.1±24.1	89.2±25.6	151.9±42.0
Gast.	HD	17	16.4±10.2	18.7±11.9	42.2±13.4	57.9±9.1	109.2±19.8
	YP	9	20.4±5.9	30.0±13.3	49.5±13.2	57.6±12.5	107.9±15.3
	OP	8	13.2±3.6	15.7±6.2	45.5±9.7	64.9±18.5	114.5±16.6
	IC	7	19.8±17.0	22.3±18.2	36.5±12.5	57.5±9.6	106.5±16.1
	AD	24	23.3±18.6	28.3±21.4	43.8±15.4	67.5±17.0	123.7±26.7
Heart	HD	18	18.9±15.1	23.4±11.2	53.2±13.2	79.6±9.9	122.7±12.8
	YP	9	10.1±7.7	28.3±17.4	50.3±14.9	80.2±8.4	112.9±12.2
	OP	7	19.5±8.9	22.8±4.7	57.0±13.2	83.8±9.5	129.6±19.9
	IC	7	19.8±11.0	22.1±7.6	66.8±13.6	89.2±15.4	155.4±17.9
	AD	22	22.7±11.6	32.4±14.8	57.6±14.0	90.5±20.4	139.7±31.9

* Age groups represented: Young chicks with nestling plumage head down under parental care (HD); young chicks under parental care (YP); older chicks under parental care (OP); independent chicks no longer under parental care (IC); adults (AD).

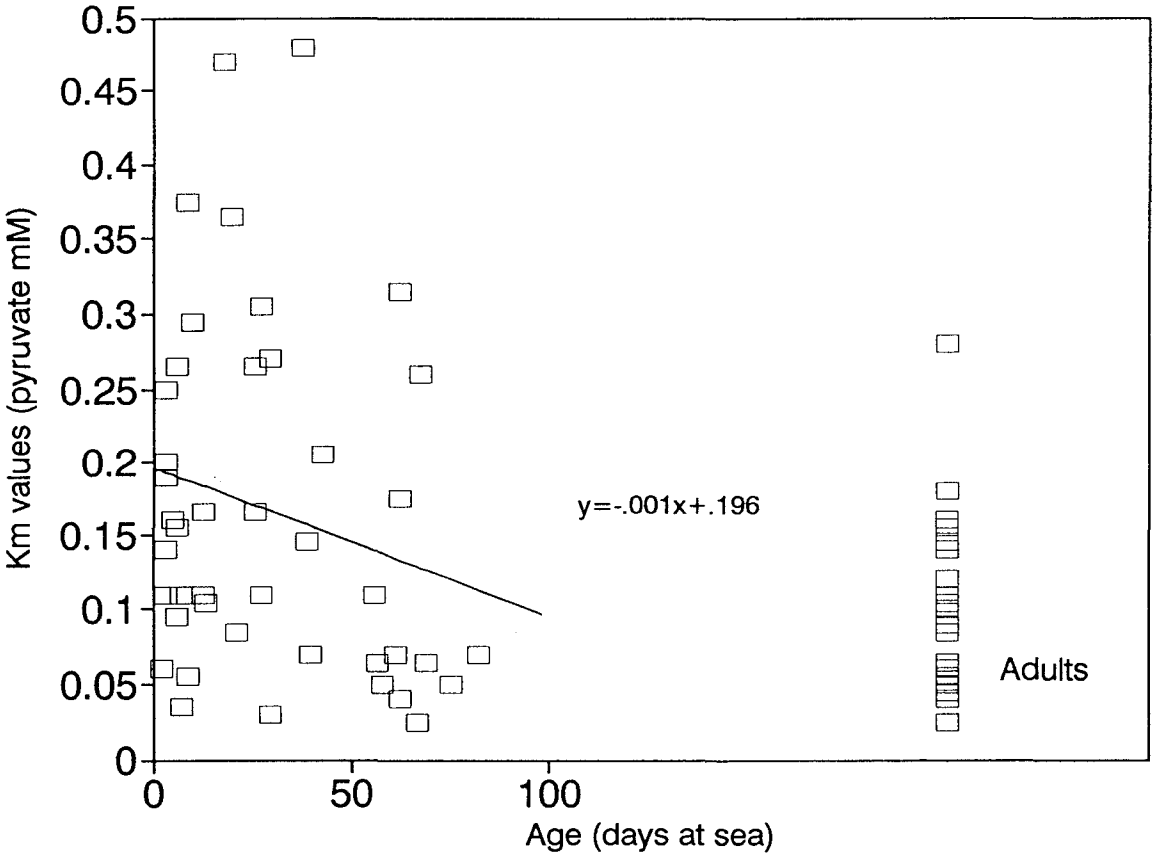


Figure 10. Pectoralis muscle LDH Km value changes with age in Common Murre chicks. Regression (chicks only) r^2=.05 (p= 0.170). Adults included for reference.

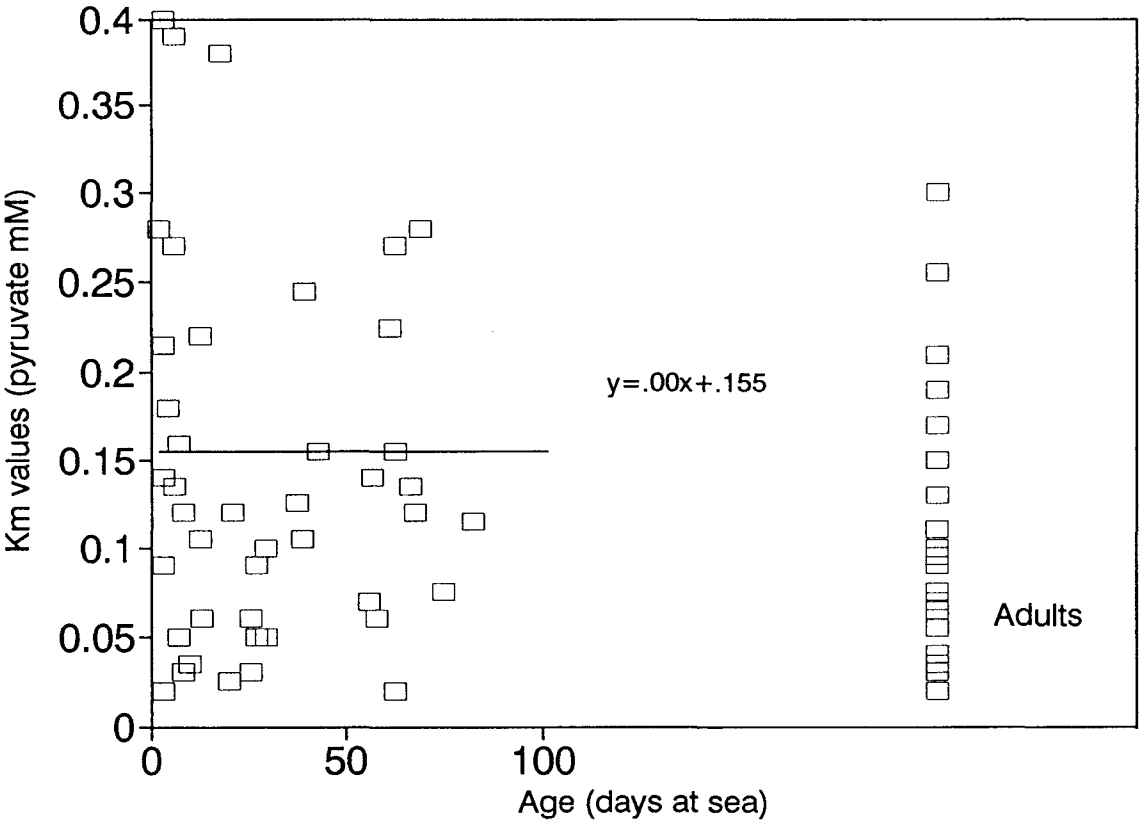


Figure 11. Gastrocnemius muscle LDH Km value changes with age in Common Murre chicks. Regression (chicks only) r^2=.01 (p= 0.507). Adults included for reference.

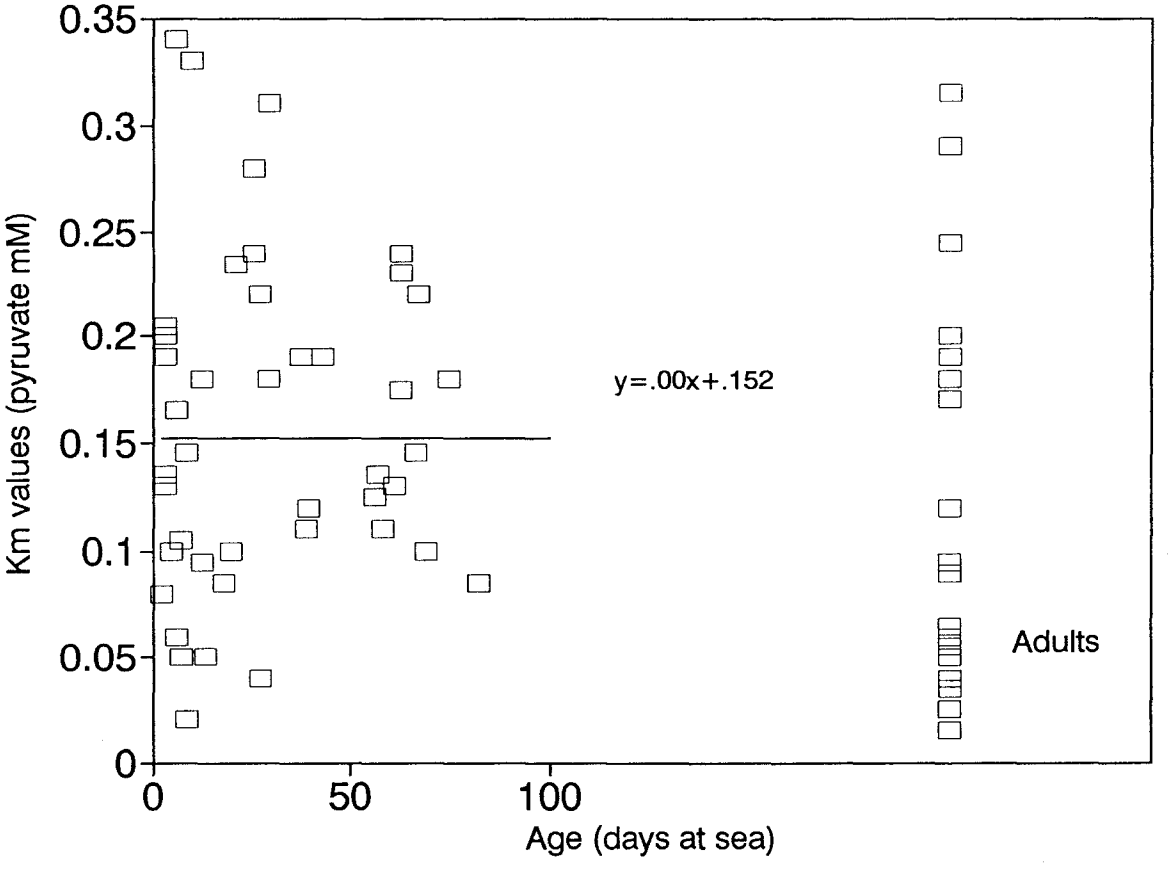


Figure 12. Heart muscle LDH Km value changes with age in Common Murre chicks. Regression (chicks only) r^2=.00 (p= 0.749). Adults included for reference.

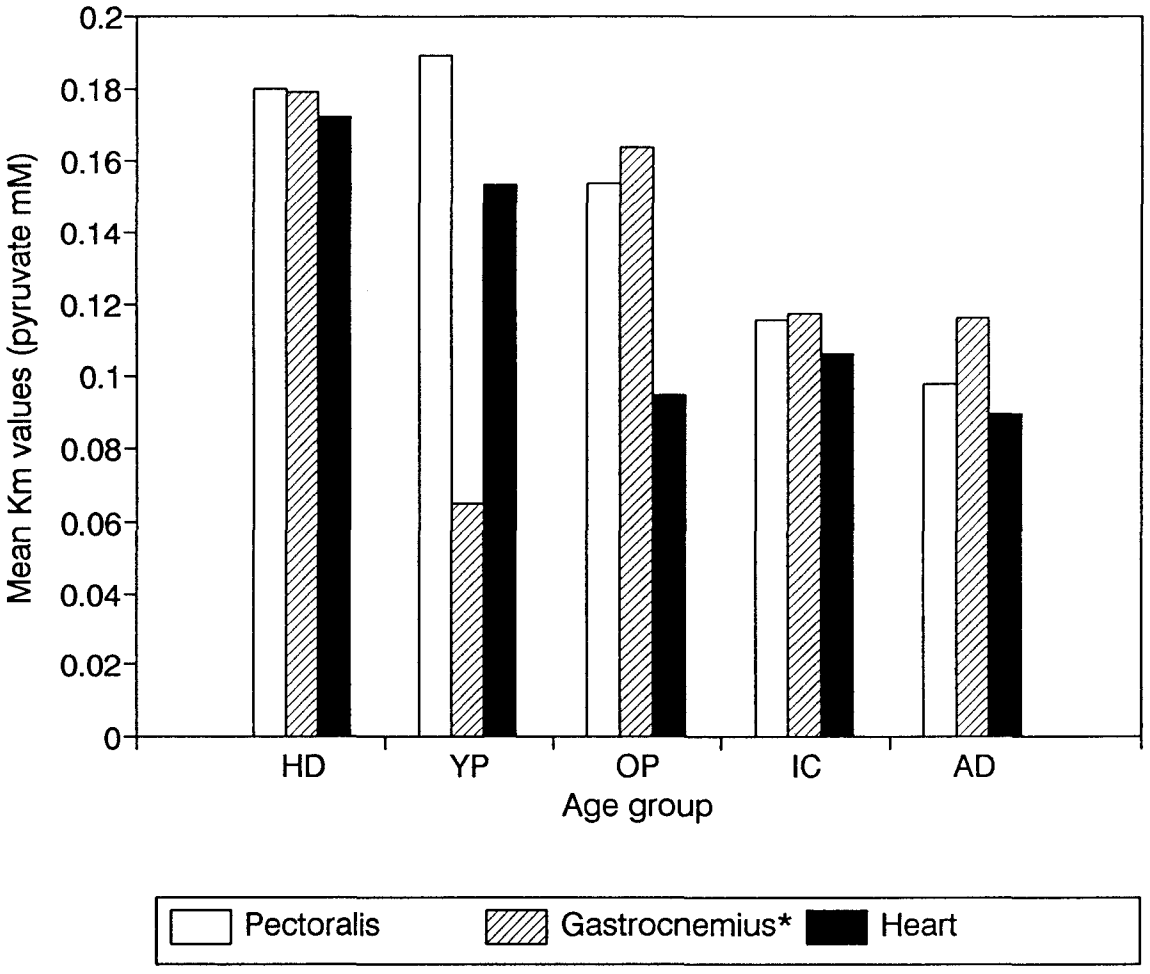


Figure 13. Mean LDH Km values of pectoralis, gastrocnemius, and heart muscle in five age groups of the Common Murre: Youngest chicks under parental care (HD), young chicks under parental care (YP), older chicks under parental care (OP), chicks no longer under parental care (IC), adults (AD).

* For gastrocnemius muscle, differences in Km values is significant at $p < 0.05$. Values summarized in Table 3.

Table 3. Mean (± standard deviation) LDH Km estimates (mM pyruvate) of pectoralis, gastrocnemius and heart muscle in five age groups of the Common Murre, with ANOVA results for differences between age groups* within each muscle type.

Muscle Tissue	p Value	Km Estimates HD (n=18)	YP (n=9)	OP (n=9)	IC (n=7)	AD (n=23)
Pect.	0.067	0.180 ±0.115	0.189 ±0.115	0.154 ±0.144	0.116 ±0.100	0.098 ±0.059
Gast.	0.026	0.179 ±0.123	0.065 ±0.032	0.164 ±0.080	0.117 ±0.067	0.116 ±0.081
Heart	0.385	0.145 ±0.087	0.184 ±0.098	0.152 ±0.058	0.160 ±0.037	0.121 ±0.084

* Age groups represented: Youngest chicks with nestling plumage head down under parental care (HD); young chicks under parental care (YP); older chicks under parental care (OP); independent chicks no longer under parental care (IC); adults (AD).

Electrophoresis

Regression analysis of LDH isozymes for the pectoralis muscle showed increases in intensity of staining for LDH 1, LDH 2, LDH 3, and LDH 4. No increase in LDH 5 intensity with maturation was observed. LDH 1 (Figure 14) showed a positive relationship with increase in age, r^2=.49 ($p<0.001$). LDH 2 (Figure 15), LDH 3 (Figure 16), and LDH 4 (Figure 17) also showed significant strong positive correlation to increasing age; r^2=.67 ($p<0.001$), r^2=.75 ($p<0.001$), and r^2=.66 ($p<0.001$), respectively. Clearly, age of murre chick is a good predictor of intensity of LDH 1, LDH 2, LDH 3, and LDH 4 in pectoralis muscle. LDH 5 (Figure 18) showed no relationship to age, r^2=.04 ($p=0.214$). Figure 19 represents the total pectoralis isozyme compositions of the five Common Murre age groups, and Table 4 summarizes the mean intensity values of those isozymes. Again, it appears that LDH 1, LDH 2, LDH 3, and LDH 4 do increase with maturation and LDH 5 does not. Additionally, there are no increases in any isozymes from IC age group chicks to adult.

Intensity of four of the five LDH isozymes in the gastrocnemius muscle showed significant increase with age: LDH 1 r^2=.18, $p<0.005$ (Figure 20); LDH 2 r^2=.24, $p=0.001$ (Figure 21); LDH 3 r^2=.35, $p<0.001$ (Figure 22); LDH 4

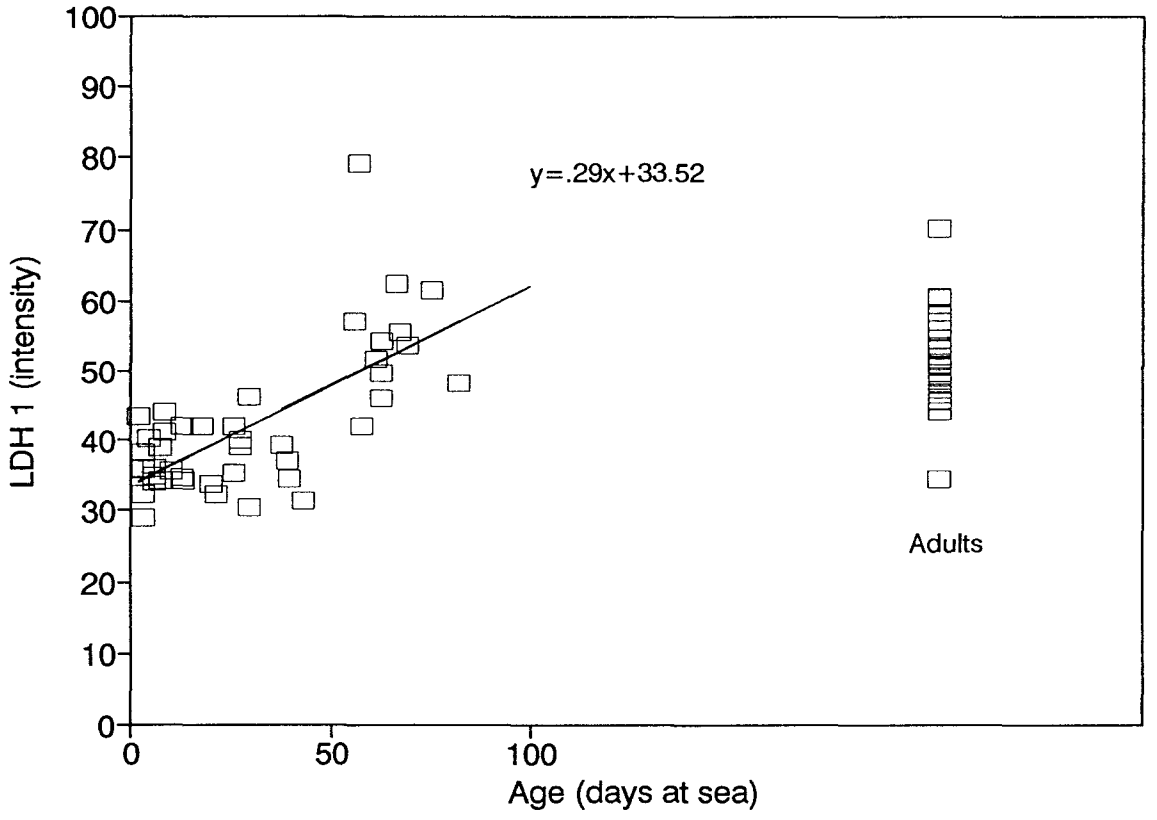


Figure 14. Pectoralis muscle LDH 1 intensity changes with age in Common Murre chicks. Regression (chicks only) r^2=.49 ($p < 0.001$). Adult values included for reference.

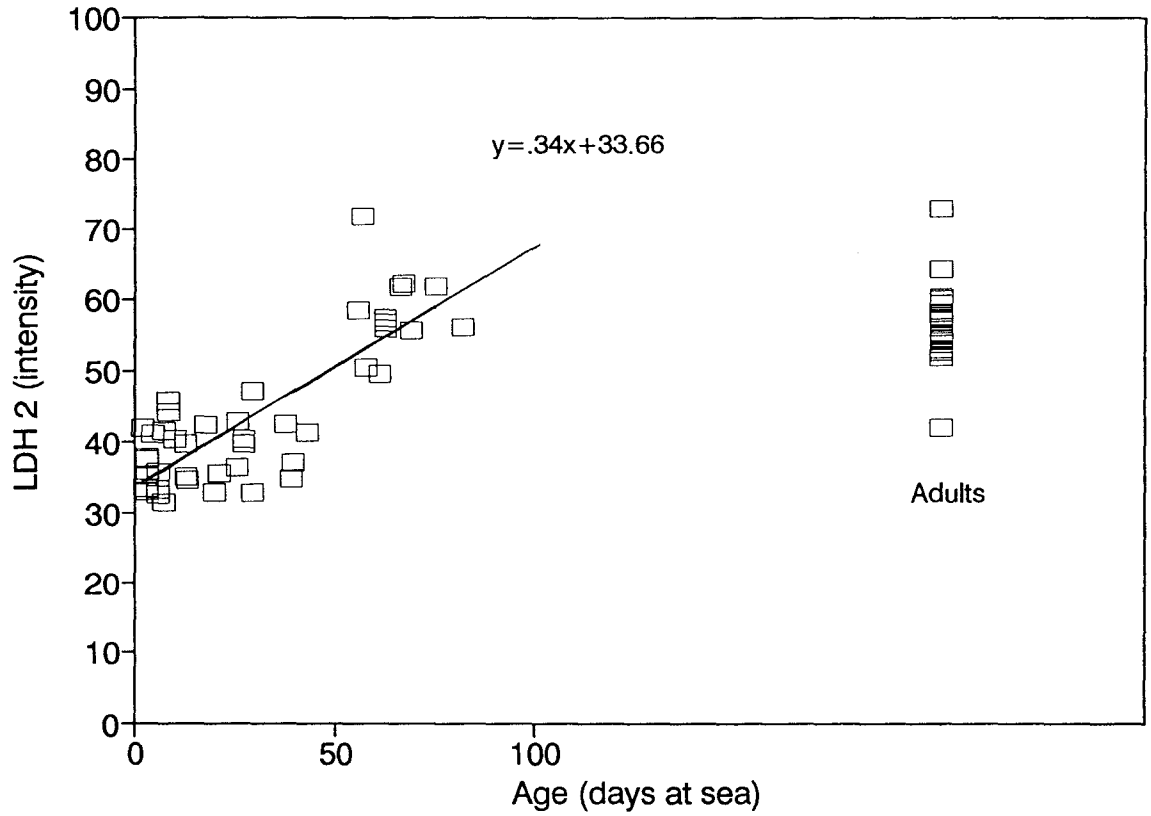


Figure 15. Pectoralis muscle LDH 2 intensity changes with age in Common Murre chicks. Regression (chicks only) $r^2=.67$ ($p< 0.001$). Adult values included for reference.

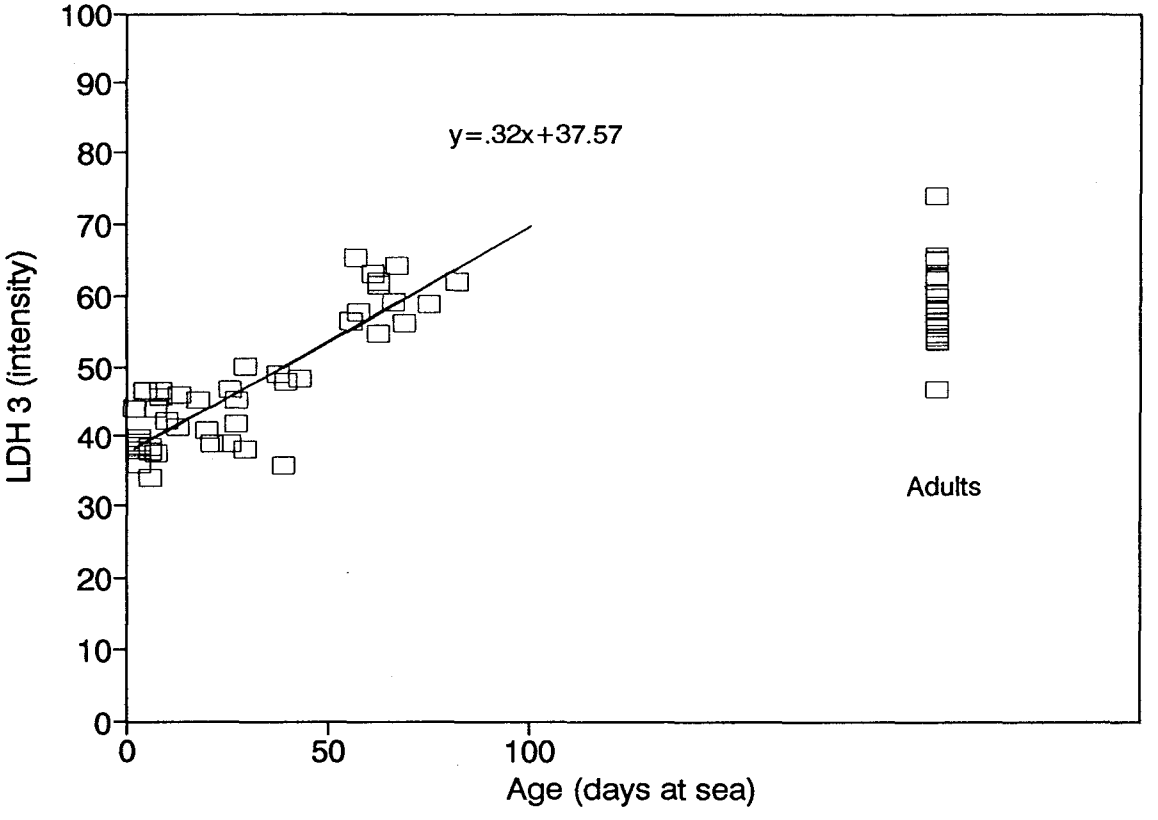


Figure 16. Pectoralis muscle LDH 3 intensity changes with age in Common Murre chicks. Regression (chicks only) r^2=.75 ($p < 0.001$). Adult values included for reference.

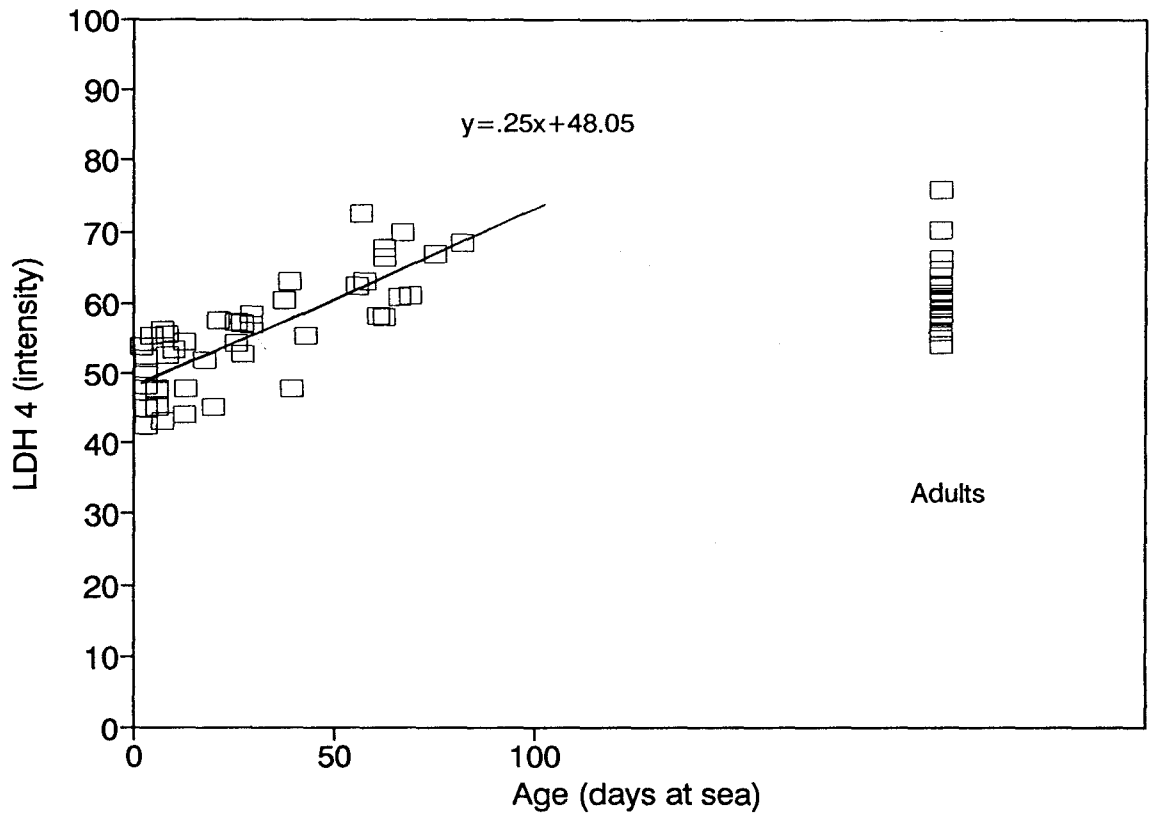


Figure 17. Pectoralis muscle LDH 4 intensity changes with age in Common Murre chicks. Regression (chicks only) r^2=.65 ($p< 0.001$). Adult values included for reference.

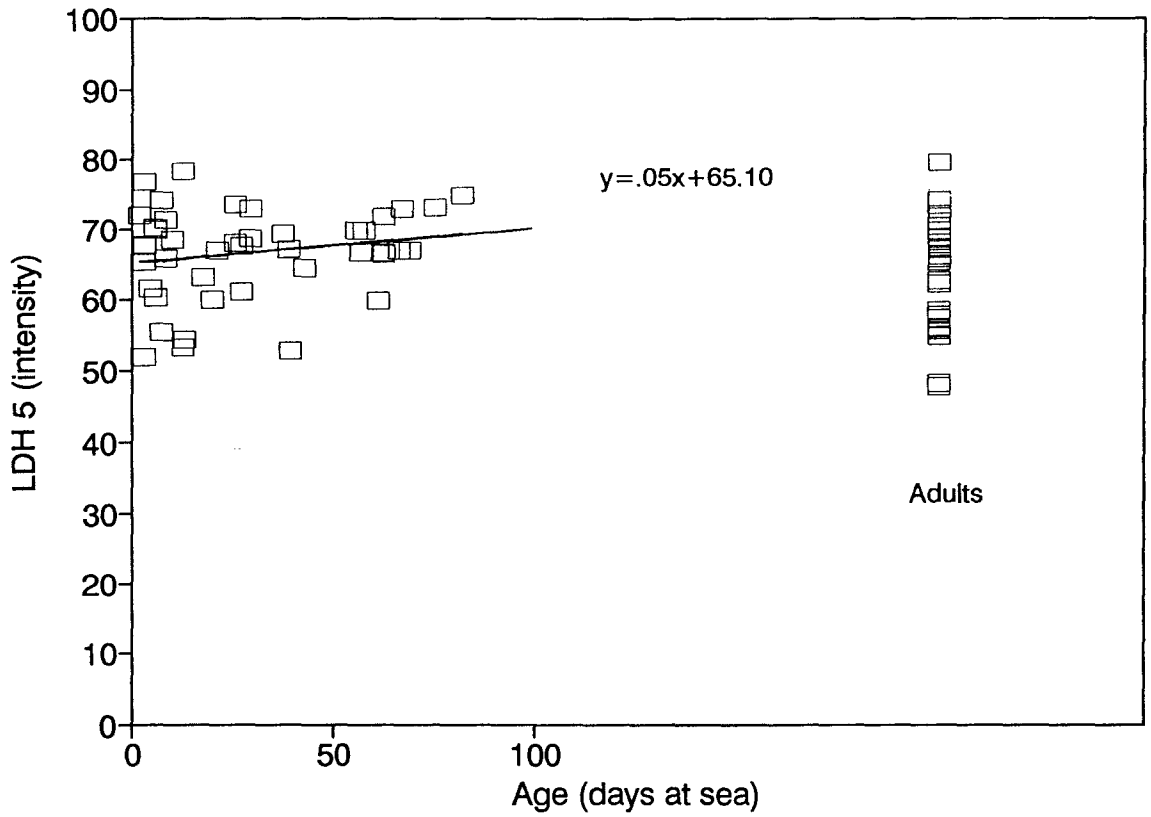


Figure 18. Pectoralis muscle LDH 5 intensity changes with age in Common Murre chicks. Regression (chicks only r^2=.04 (p= 0.214). Adult values included for reference.

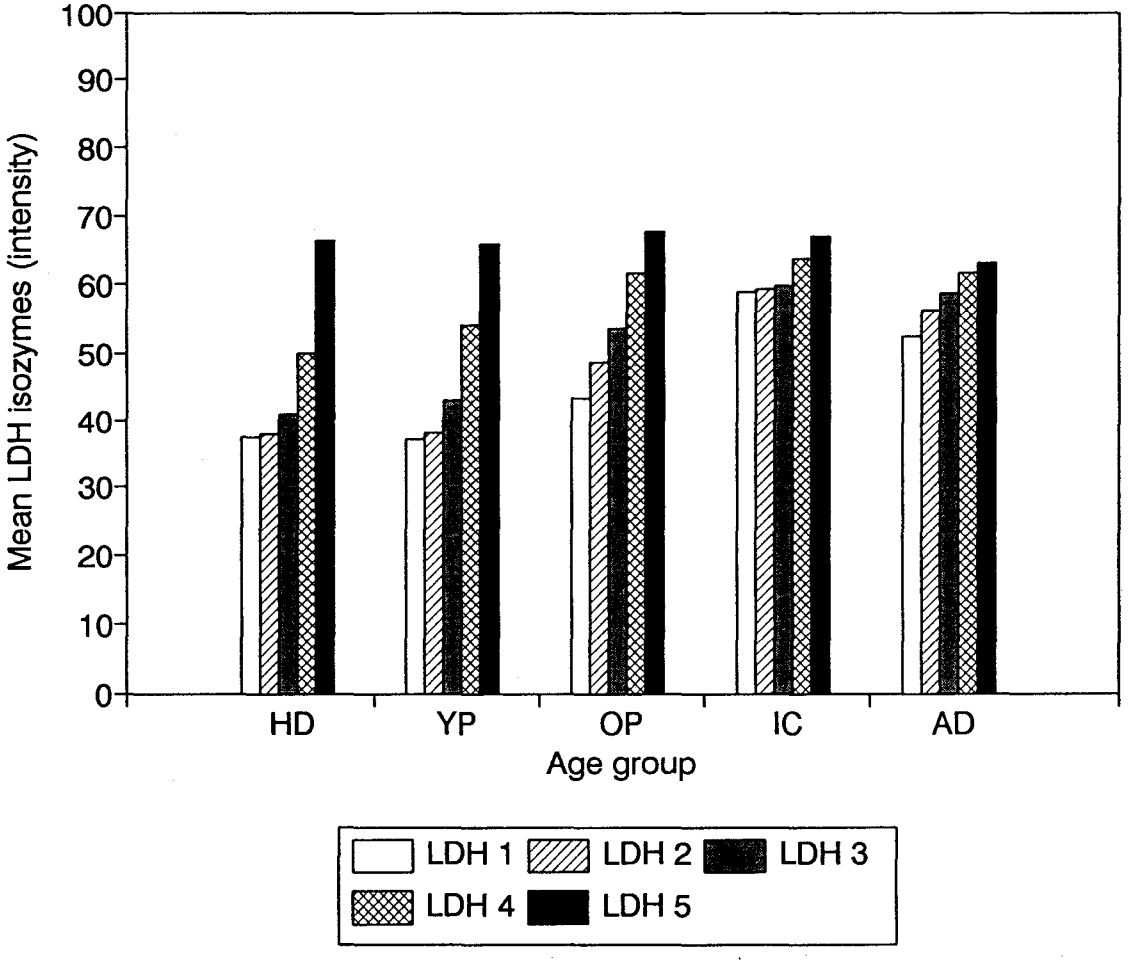


Figure 19. Pectoralis muscle LDH isozyme intensity in five age groups of the Common Murre: Youngest chicks under parental care (HD); young chicks under parental care (YP); older chicks under parental care (OP); chicks no longer under parental care (IC); adults (AD).

Table 4. Mean (± standard deviation) intensity values of LDH isozymes in pectoralis, gastrocnemius and heart muscle in five age groups* of the Common Murre.

Muscle Tissue	Age Group	Sample Size (n)	Isozyme Intensity LDH 1	LDH 2	LDH 3	LDH 4	LDH 5
Pect.	HD	18	37.4±4.0	37.9±4.3	40.9±3.7	49.9±4.6	66.2±7.5
	YP	9	37.1±5.0	38.1±4.7	42.9±4.2	54.2±4.8	65.9±6.2
	OP	9	43.4±8.6	48.6±9.8	53.7±9.2	61.7±6.8	67.7±6.4
	IC	7	58.9±10.6	59.4±6.8	59.9±3.6	63.8±5.3	67.0±3.9
	AD	23	52.5±7.3	56.3±5.5	58.7±5.7	61.8±5.0	63.1±8.1
Gast.	HD	18	60.3±8.4	55.9±7.4	53.7±4.3	55.7±6.1	58.9±8.6
	YP	9	58.1±7.4	55.8±5.2	55.6±3.0	56.4±7.7	57.7±8.4
	OP	9	66.3±7.7	63.1±7.9	60.0±5.8	60.0±6.6	59.8±8.0
	IC	7	68.3±9.2	61.8±7.5	57.7±2.8	60.5±6.7	62.0±5.5
	AD	23	63.1±6.3	57.7±6.0	55.4±5.2	58.8±5.2	61.6±6.9
Heart	HD	17	80.6±7.7	34.5±34.5	35.4±24.3	36.9±14.6	36.4±3.9
	YP	9	74.6±5.6	65.7±9.8	38.1±31.1	50.0±11.2	40.3±17.1
	OP	9	81.0±7.4	13.5±26.8	16.8±25.3	13.9±21.0	21.2±20.3
	IC	7	82.5±5.3	42.1±39.5	31.2±29.3	32.6±22.3	33.2±14.9
	AD	22	81.8±4.6	19.4±32.6	23.0±28.5	15.9±21.6	25.1±19.8

* Age groups represented: Youngest chicks with nestling plumage head down under parental care (HD); young chicks under parental care (YP); older chicks under parental care (OP); independent chicks no longer under parental care (IC); adults (AD).

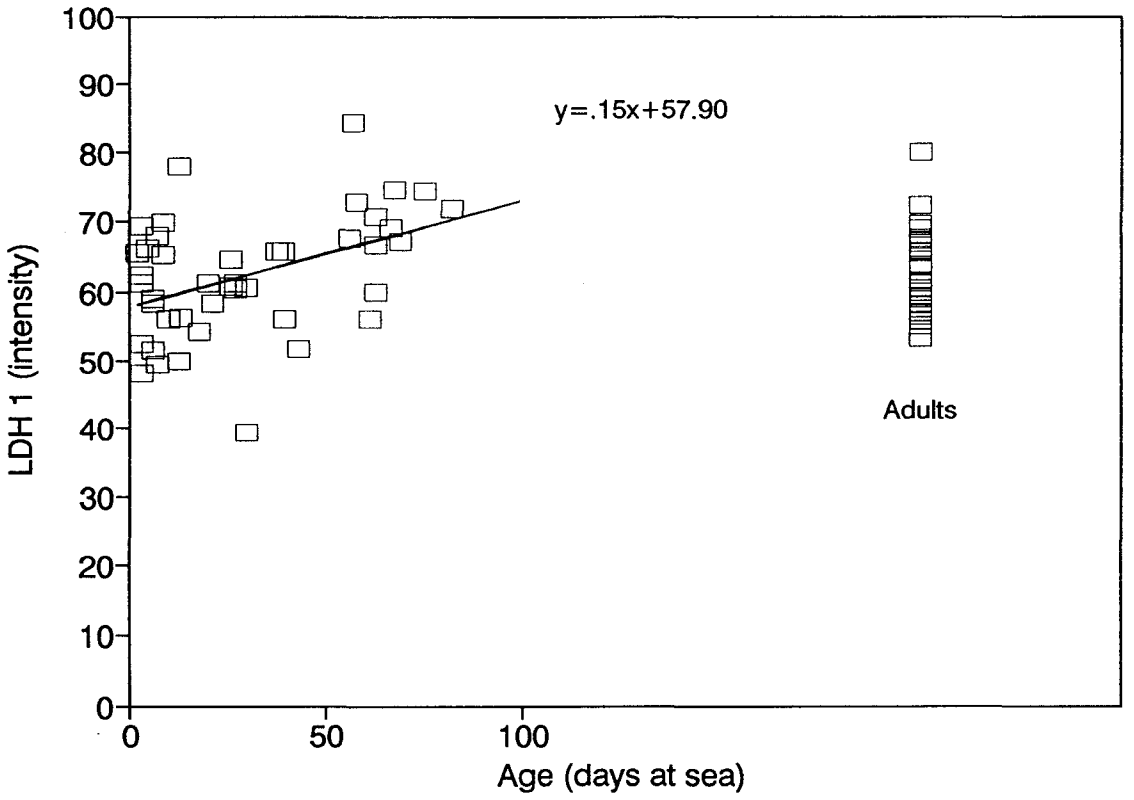


Figure 20. Gastrocnemius muscle LDH 1 intensity changes with age in Common Murre chicks. Regression (chicks only) r^2=.18 ($p< 0.005$). Adult values included for reference.

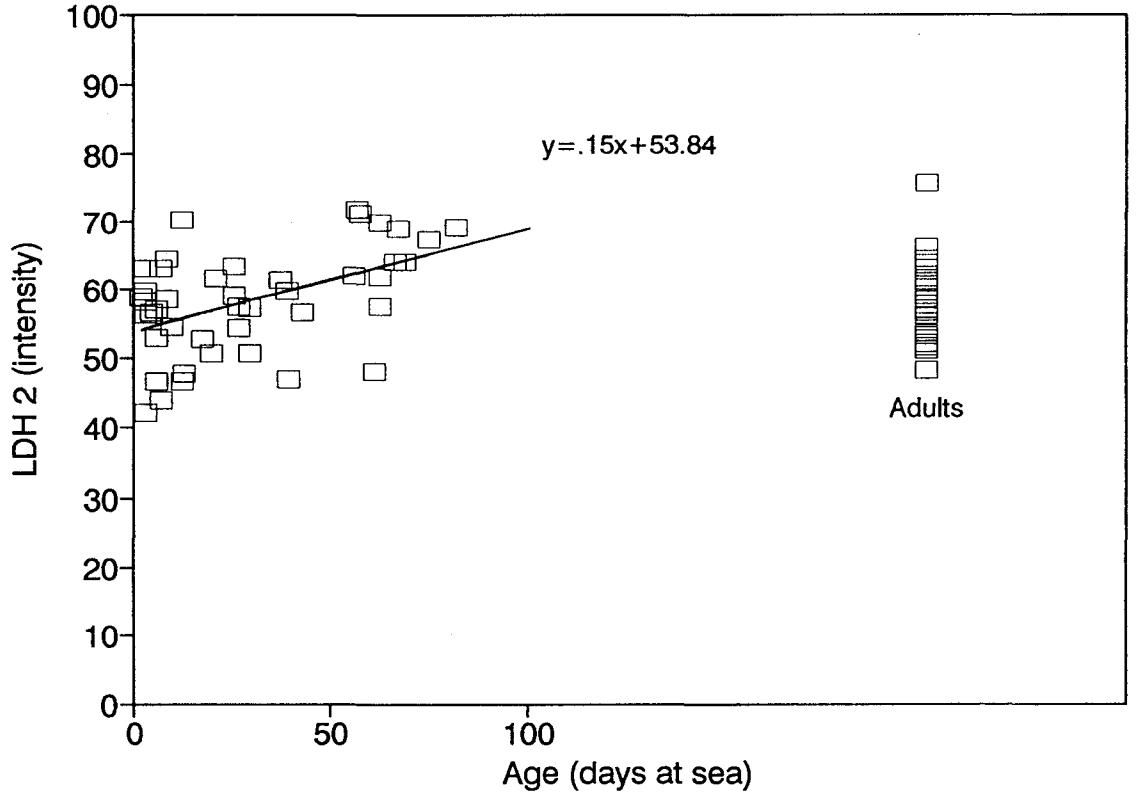


Figure 21. Gastrocnemius muscle LDH 2 intensity changes with age in Common Murre chicks. Regression (chicks only) r^2=.24 ($p < 0.005$). Adult values included for reference.

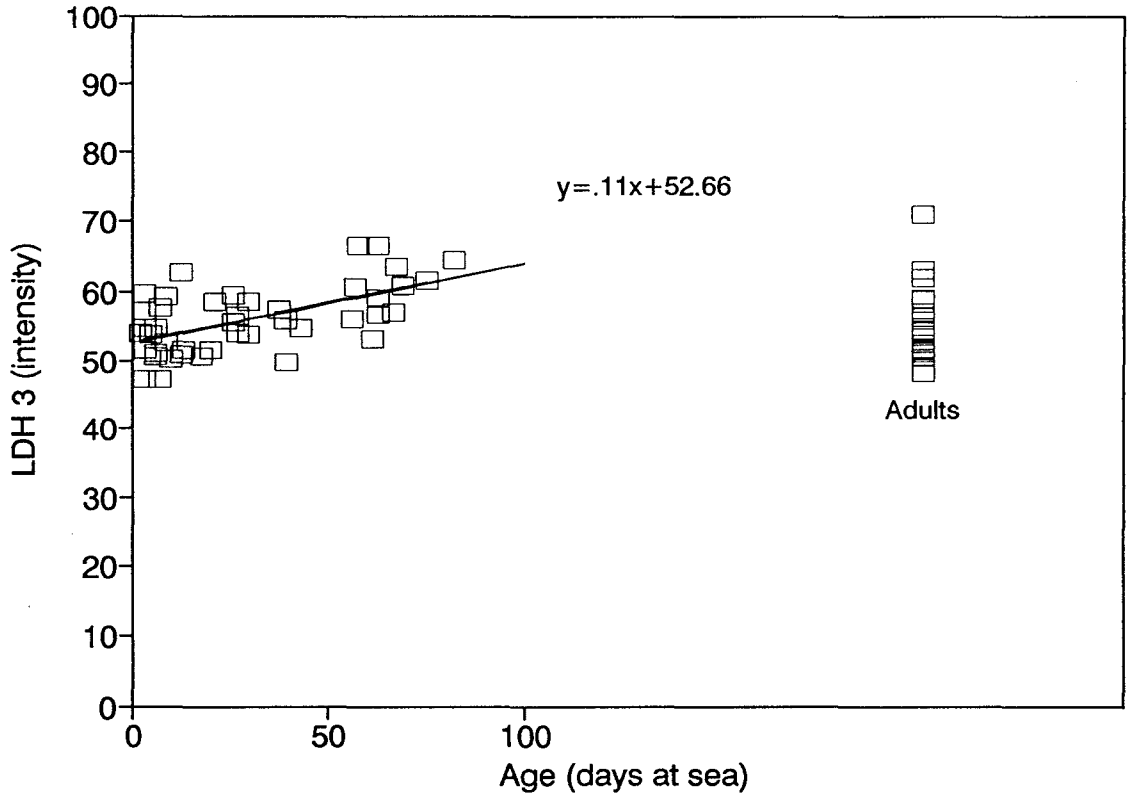


Figure 22. Gastrocnemius muscle LDH 3 intensity changes with age in Common Murre chicks. Regression (chicks only) r^2=.35 ($p < 0.001$). Adult values included for reference.

r^2=.17, p<0.01 (Figure 23), however only 17-35% of the variation in these isozymes can be explained by age of murre chick. LDH 5 showed no significant change in intensity with age (Figure 24, r^2=.07, p>0.05). Figure 25 depicts total gastrocnemius LDH isozyme compositions in the five Common Murre age groups (summarized in Table 4) illustrating the lack of change in all five LDH isozymes with maturation.

Heart muscle LDH isozyme regressions showed no relationship of the five LDH isozymes with maturation: LDH 1 r^2=.02, p=0.337 (Figure 26); LDH 2 r^2=.06, p=0.261 (Figure 27); LDH 3 r^2=.04, p=0.361 (Figure 28); LDH 4 r^2=.05, p= 0.212 (Figure 29); LDH 5 r^2=.03, p=0.33 (Figure 30). Because LDH 2, LDH 3, LDH 4, and LDH 5 do not always occur in the heart muscle, regressions of LDH isozyme by age used only intensity values greater than zero; i.e., when the isozyme band was not present, the resulting intensity value of zero was not included in the regression analysis. The effect of including intensity=0 values is illustrated in Figure 31; the variability in mean LDH 2, LDH 3, LDH 4, and LDH 5 intensity between age groups reflects not differences in amount of LDH but rather differential presence and absence in group samples. Thus, regressions showed that in fact when present, amount of LDH is <u>not</u> correlated with age. Figure 32 complements Figure 31 by displaying the frequency of occurrence of heart isozyme in the five Common Murre age groups. A Chi-square (X^2) contingency table analysis was

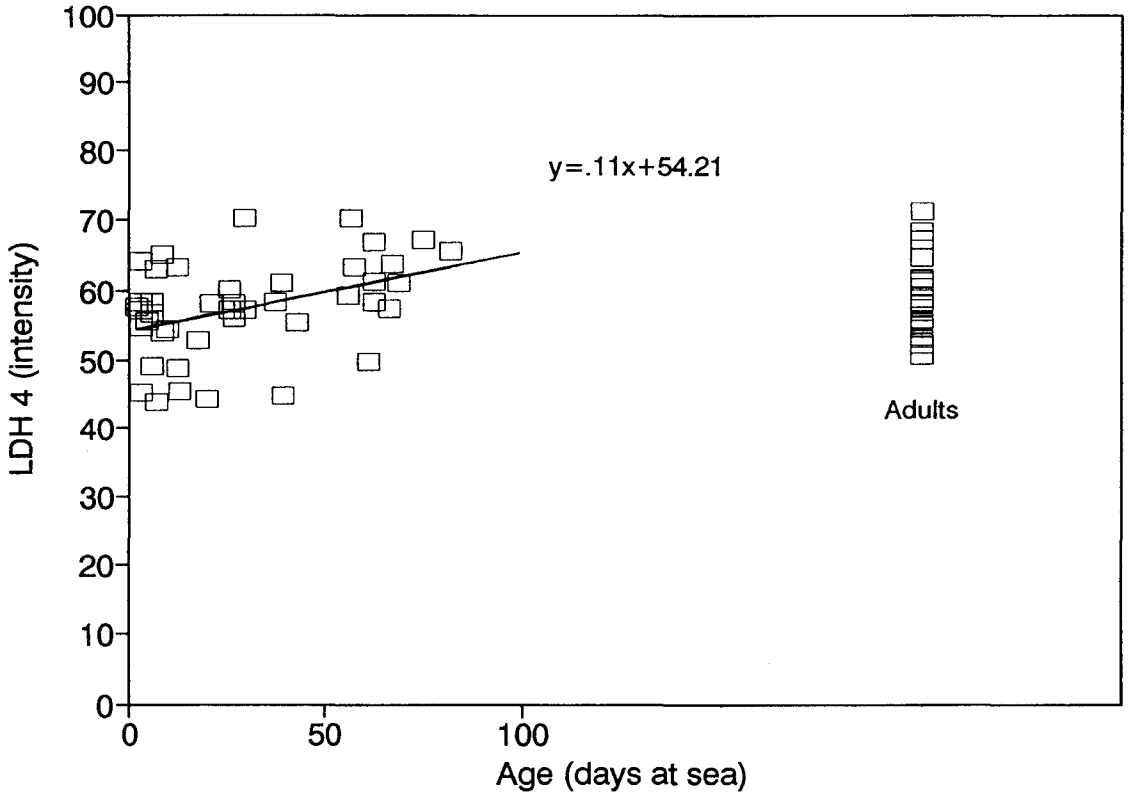


Figure 23. Gastrocnemius muscle LDH 4 intensity changes with age in Common Murre chicks. Regression (chicks only) r^2=.17 ($p< 0.01$). Adult values included for reference.

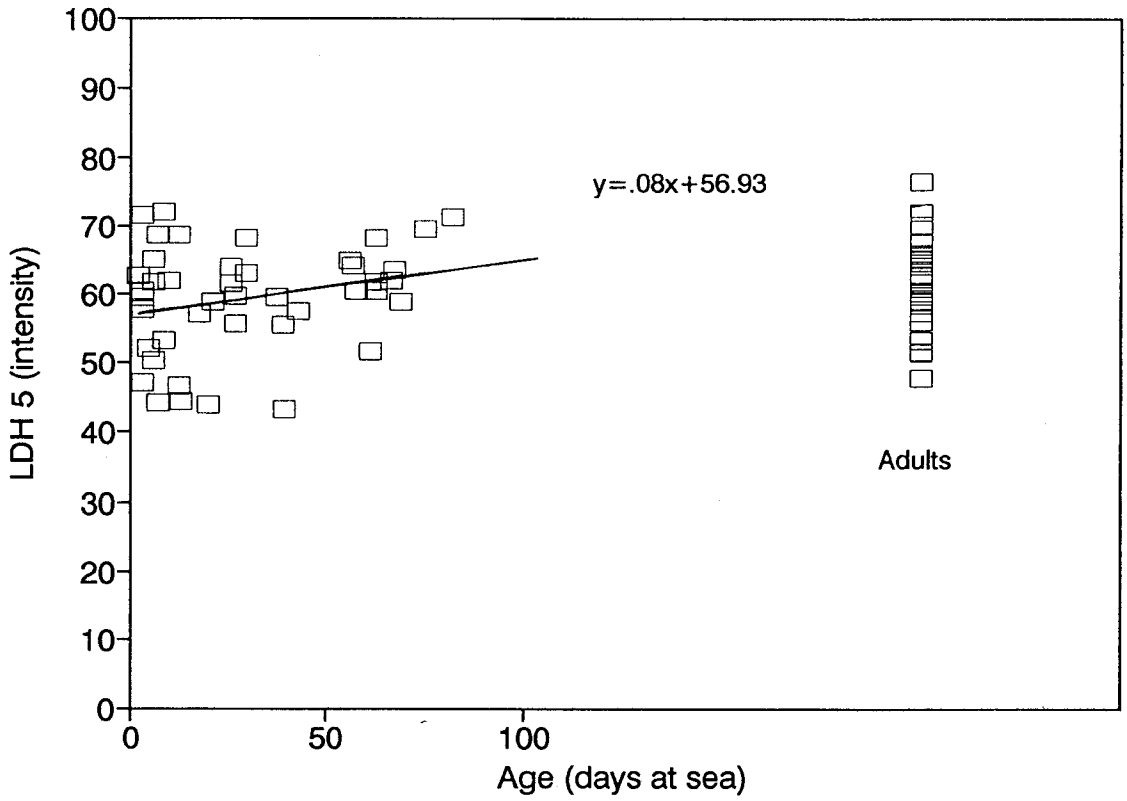


Figure 24. Gastrocnemius muscle LDH 5 intensity changes with age in Common Murre chicks. Regression (chicks only) r^2=.07 (p= 0.098). Adult values included for reference.

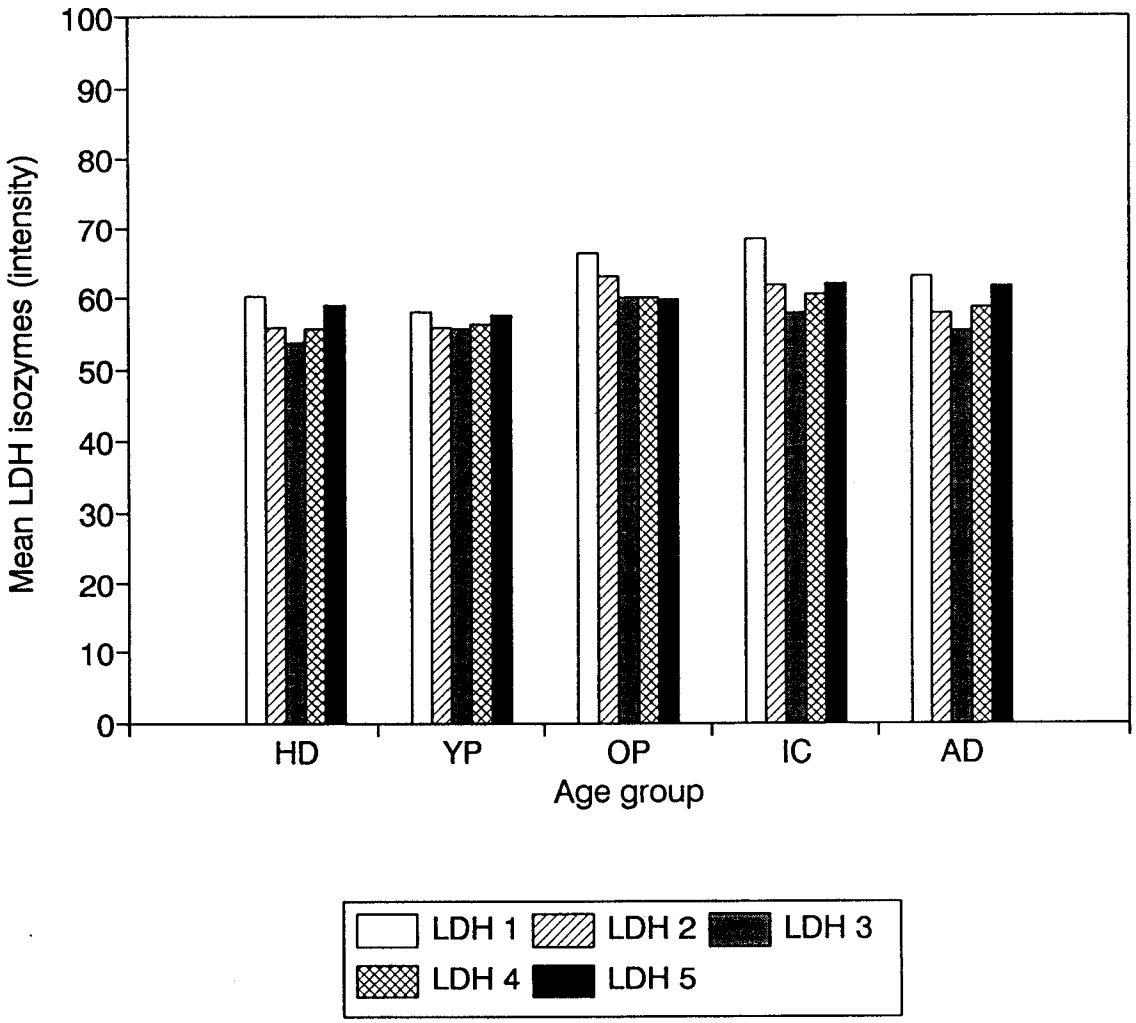


Figure 25. Gastrocnemius muscle LDH isozyme intensity in five age groups of the Common Murre: Youngest chicks under parental care (HD); young chicks under parental care (YP); older chicks under parental care (OP); chicks no longer under parental care (IC); adults (AD).

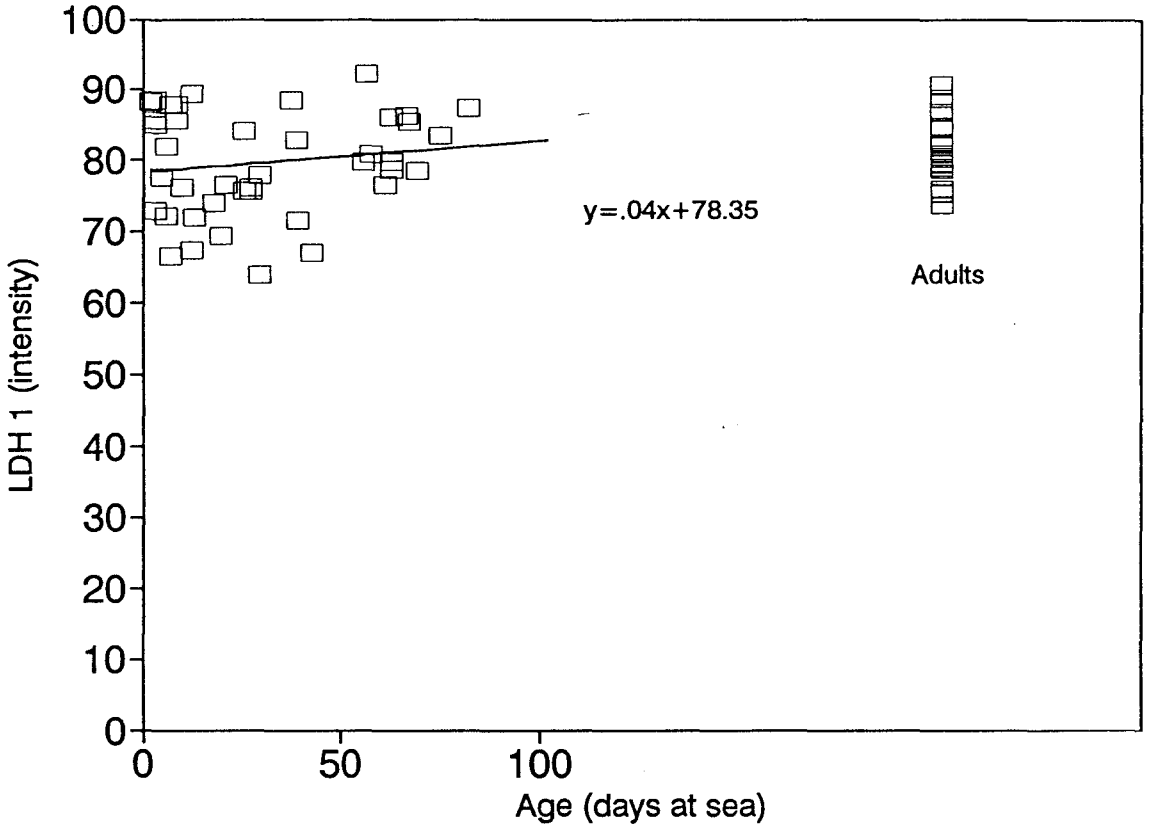


Figure 26. Heart muscle LDH 1 intensity changes with age in Common Murre chicks. Regression (chicks only) r^2=.02 (p= 0.337). Adult values included for reference.

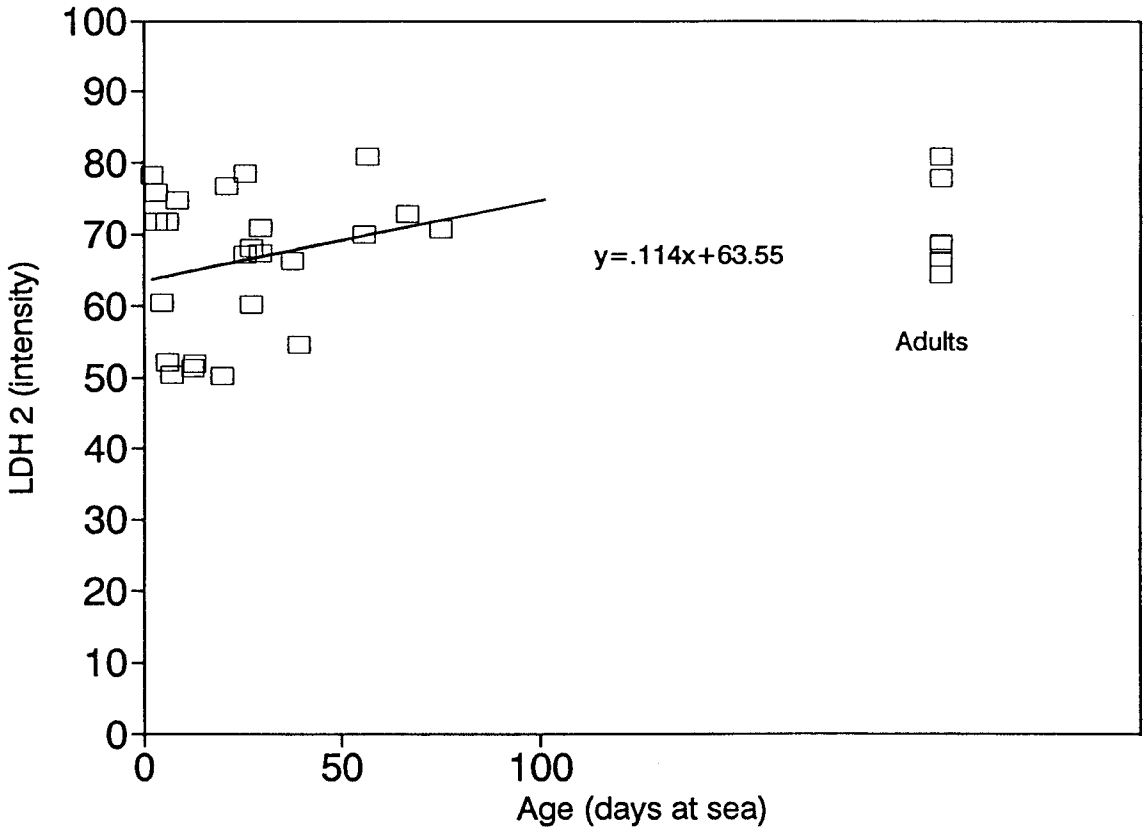


Figure 27. Heart muscle LDH 2 intensity changes with age in Common Murre chicks. Regression (chicks only) r^2=.06 (p= 0.261). Adult values included for reference.

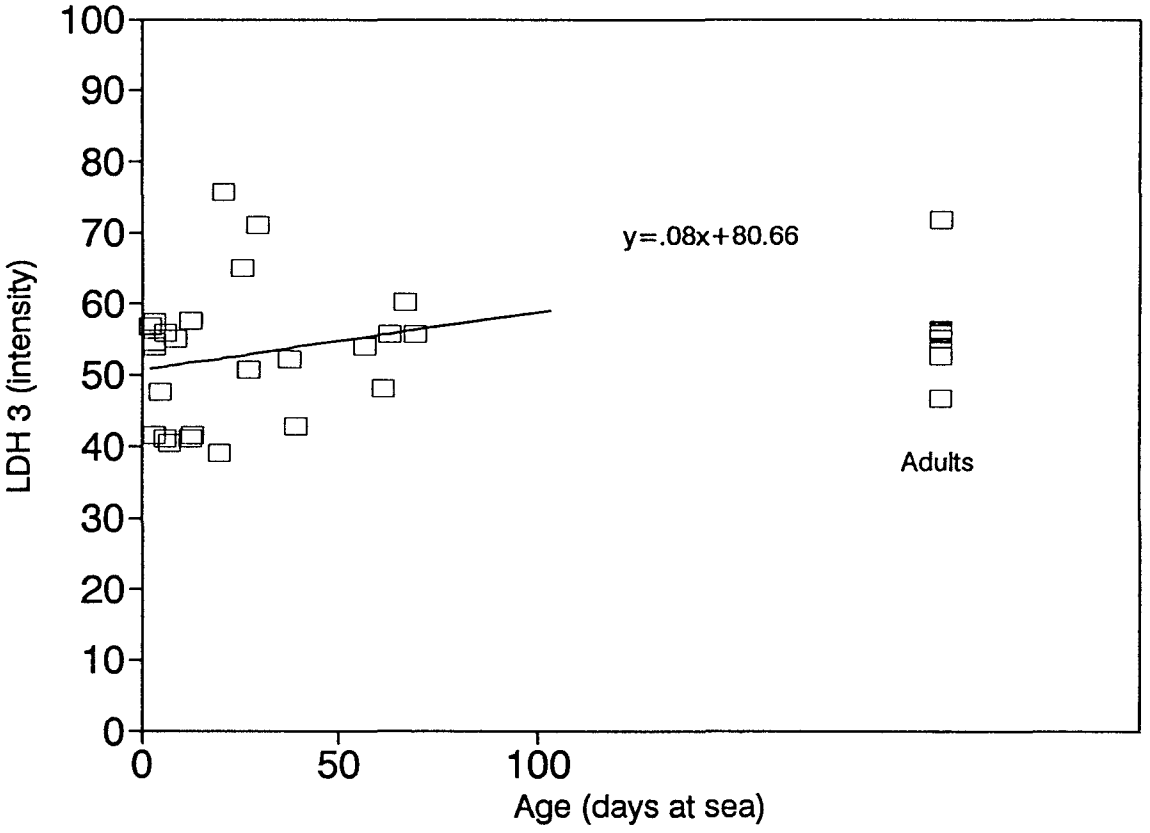


Figure 28. Heart muscle LDH 3 intensity changes with age in Common Murre chicks. Regression (chicks only) r^2=.04 (p= 0.361). Adults values included for reference.

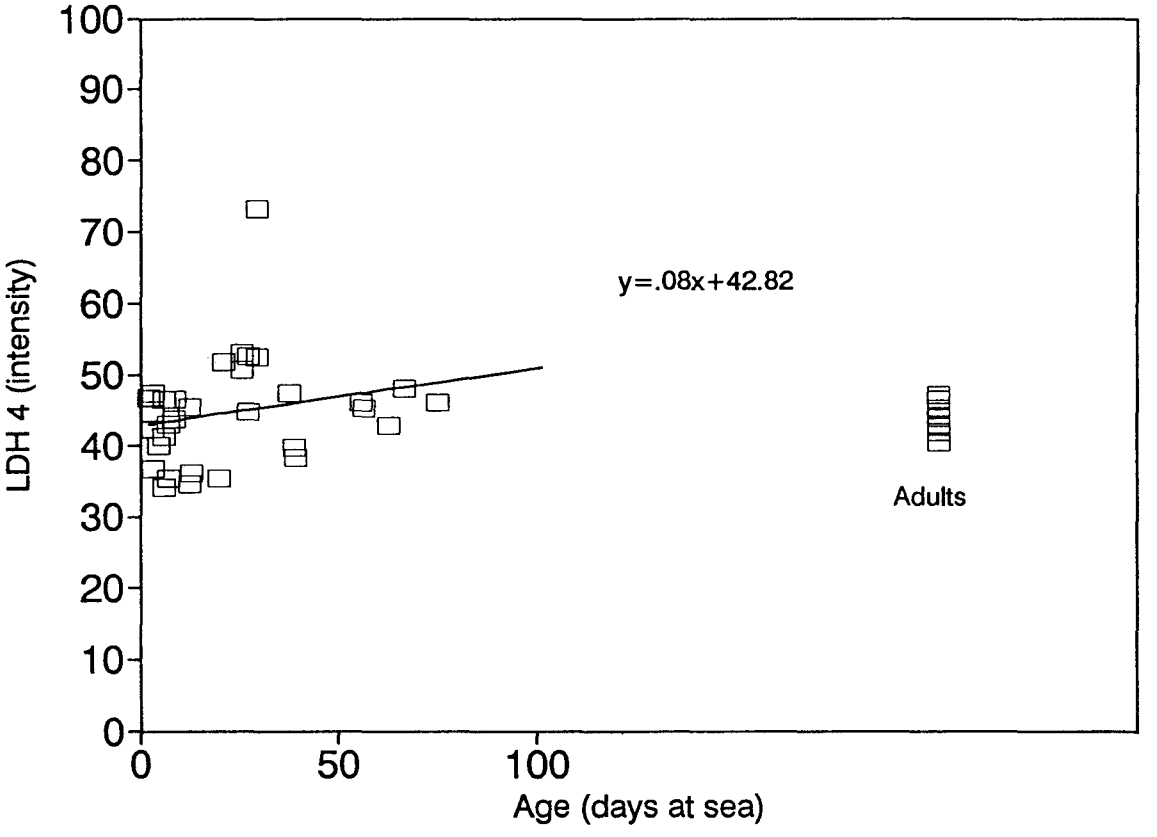


Figure 29. Heart muscle LDH 4 intensity changes with age in Common Murre chicks. Regression (chicks only) r^2=.05 (p= 0.212). Adult values included for reference.

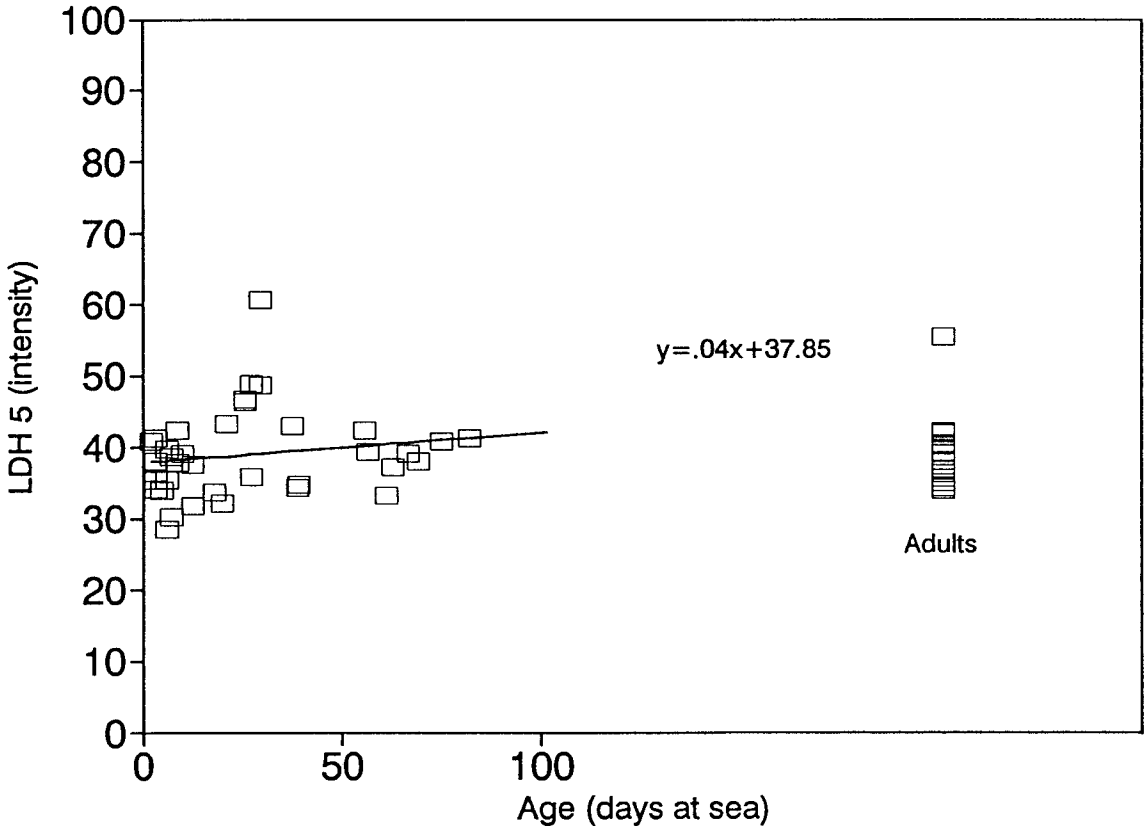


Figure 30. Heart muscle LDH 5 intensity changes with age in Common Murre chicks. Regression (chicks only) r^2=.03 (p= 0.330). Adult values included for reference.

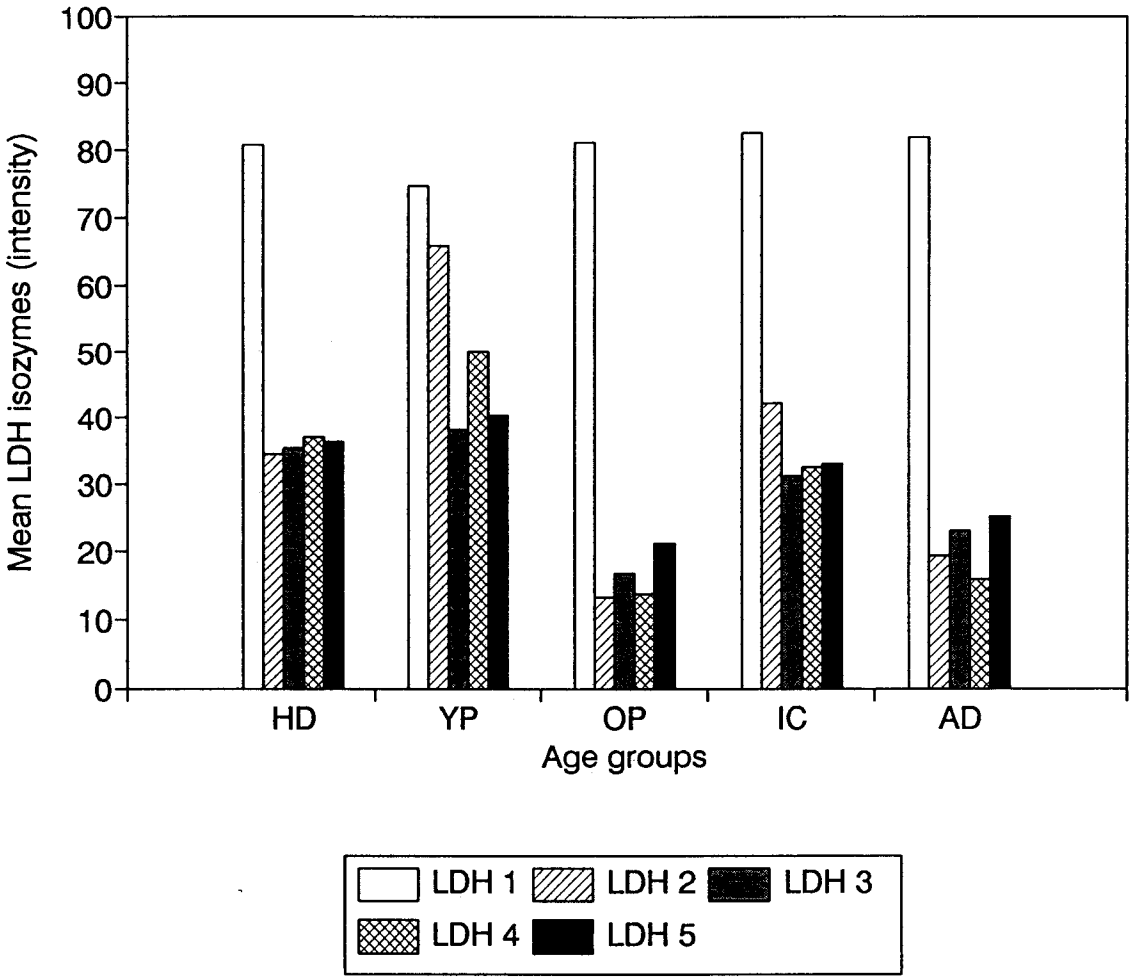


Figure 31. Heart muscle LDH isozyme intensity in five age groups of the Common Murre: Youngest chicks under parental care (HD); young chicks under parental care (YP); older chicks under parental care (OP); chicks no longer under parental care (IC); adults (AD).

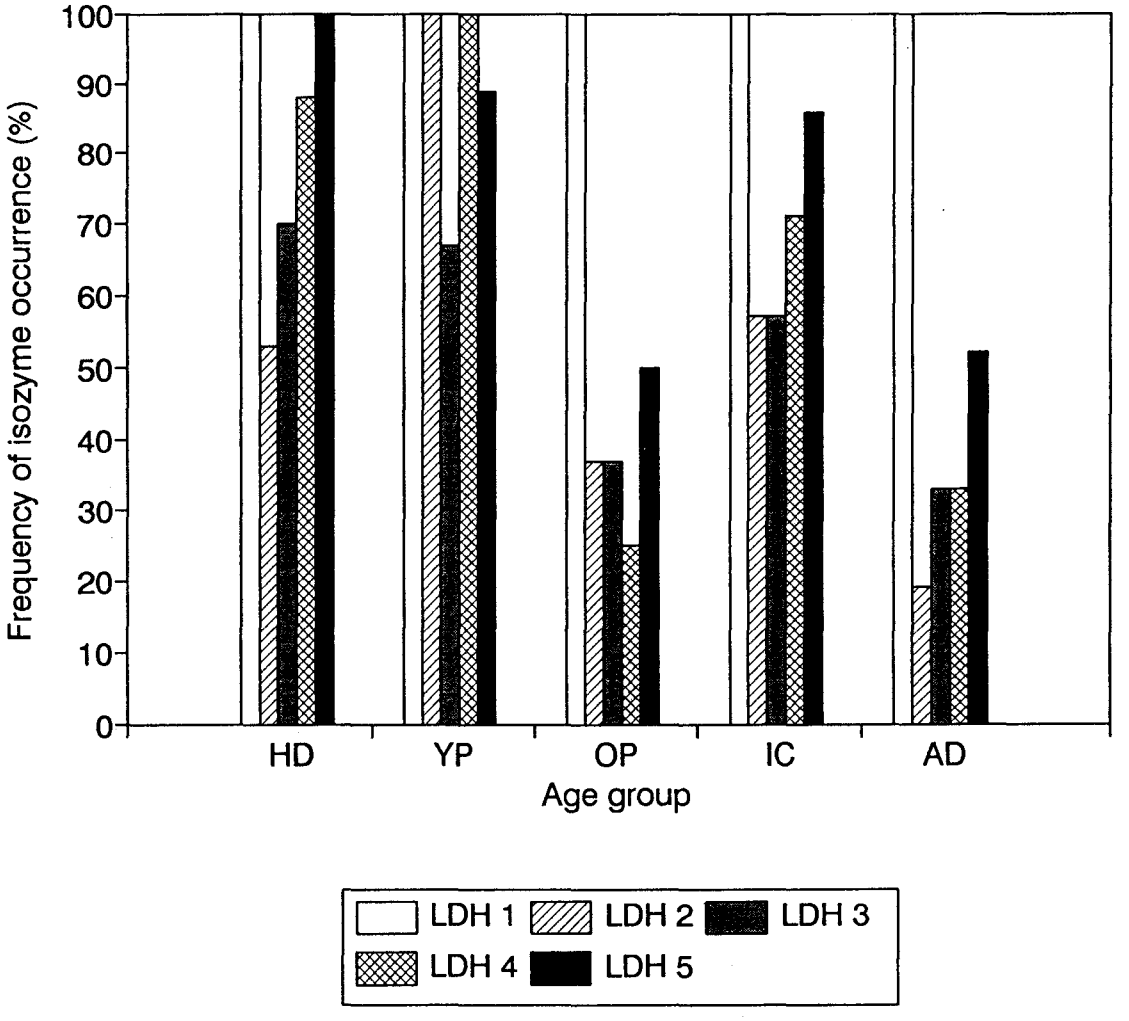


Figure 32. Heart muscle LDH isozyme occurrence in five age groups of the Common Murre: Youngest chicks under parental care (HD); young chicks under parental care (YP); older chicks under parental care (OP); chicks no longer under parental care (IC); adults (AD).

performed on these heart isozymes to test frequency of occurence among age groups. The X^2 results showed no significant difference among Common Murre age groups for LDH 1 and LDH 3 ($p>0.05$); i.e., LDH 1 and LDH 3 occur with the same frequency among age groups. However, the difference in the frequency of occurrence of LDH 3 among age groups shows a X^2 value that is nearly significant, $X^2= 6.6$ ($p=0.05$). LDH 2 ($p<0.01$), LDH 4 ($p<0.001$), and LDH 5 ($p<0.01$) showed significant differences in frequency of occurrence among Common Murre age groups. As apparent in Figure 32, LDH 2, LDH 3, LDH 4, and LDH 5 occur most frequently in the HD and YP age groups.

CHAPTER IV

DISCUSSION

Blood Parameters

Diving marine birds and mammals generally have high hemoglobin concentrations ([Hb]) and hematocrits (Hct) compared to nondiving species (Lenfant, et. al., 1969; Butler and Jones, 1982). An increase in the concentration of red blood cells in the circulation maximizes the Hb concentration in the blood and enhances the blood oxygen stores by increasing the oxygen carrying capacity (Snyder, 1983; Hochachka and Somero, 1984). When comparing blood of nonaquatic versus aquatic birds, [Hb] and Hct are generally greater in aquatic birds (Bond and Gilbert, 1958; Lenfant, et. al., 1969). As shown in Figures 1 and 2, both [Hb] and Hct increase significantly during maturation of the Common Murre chick.

As was seen by Snyder (1983), blood oxygen stores appear to be elevated only in the stage of maturity at which a marine mammal species is actively diving. Adult murre blood parameter values (Table 1) are very similar to independent chick (IC) values, indicating that the blood

oxygen stores are near adult values when the chick is abandoned by its parent and must actively forage for itself. Although blood oxygen store levels continually increase with age, peak [Hb] and Hct levels appear to be reached concurrently with an active diving regime.

When compared to other seabird species, the Common Murre YP age group showed [Hb] and Hct values much lower than Pigeon Guillemot (Cepphus columba) fledglings of similar age (Haggblom, 1987). This disparity in oxygen carrying capacity reflects differences in the developmental patterns between the two seabirds. Pigeon Guillemots are fed in the nest until slightly less than adult weight and then are abandoned by the adults (Sealy, 1973); therefore, at the fledgling stage the Pigeon Guillemot must be capable of diving to forage for itself. In contrast Common Murre chicks are fed at the nest until one-fifth of adult weight, then leave the colony to complete final growth at sea (Sealy, 1973). Their at-sea development is more protracted than the guillemot's, thus the murre chick doesn't attain near adult blood parameter values until much older. Pigeon Guillemot fledglings weigh an average of 411 grams (Sealy, 1973), whereas Common Murre chicks of the same age in this study weighed an average of 622 grams. Thus, these differences in blood parameters may also be due to the two species blood volumes relative to body mass at their respective stages of development. Murre chicks in the YP

age group have [Hb] and Hct values similar to the Gentoo penguin (Pygoscelis papua) and Adelie penguin (P. adeliae) chicks of comparable age (Milsom, et. al., 1973). Given the increase in oxygen storage capacity as murres mature, and the fact that in other diving vertebrates, such as small cetaceans, there is a consistent trend between depth to which the species usually dives and degree to which the blood oxygen stores are elevated (Lenfant, 1969), these blood parameters might seem to be a useful index of a species diving ability. For example, penguins, adapted for exclusively aquatic flight (Butler and Jones, 1982), are capable of diving to depths of 70m-265m for as long as 5-15 minutes (Conray and Twelves, 1972; Kooyman, et. al., 1982). Adult murre blood parameter values are relatively close to those of adult penguins, and the murre can dive to depths reached by several species of penguins, albeit for shorter duration. However, Pigeon Guillemot [Hb] and Hct values illustrate a caveat in any such generalization; they are higher than those of both penguins and Common Murres, yet the guillemot appears to be comparatively limited in its diving ability. The Pigeon Guillemot has been observed diving for 20 seconds at 10m (Haggblom, 1987). A close relative, the Black Guillemot (Cepphus grylle), can dive to depths of 35-45m for 112 seconds (Piatt and Nettleship, 1985). One possible reason for Pigeon Guillemots deviating from the trend of increased [Hb] and Hct and increased

diving abilities may be due to use of extended aerial flight.

Only birds that are very active fliers have higher [Hb] and Hct values than diving birds (Table 5). Aerial flight requires a large supply of oxygen to the tissues, supported by high ventilation (Lenfant, et. al., 1969; Butler, 1991). According to Butler (1991), in birds of similar mass, the energy consumed during aerial flight is greater than during aquatic flight. Moreover, strong fliers, such as the pigeon (Columbia livia) and hummingbird (Melanotrochilus fuscus) may have vascular adaptations similar to diving birds. Therefore, assessing the adaptive significance in terms of diving ability and of blood repiratory parameters is complicated by the fact that the characteristics that would enhance diving ability are similar to those that would allow for any type of sustained exercise. Further, other physiological characteristics of the blood, such as buffering capacity and oxygen affinity likely enhance ability for aerial and subaquatic flight (Lenfant, et. al., 1969).

Table 5 summarizes the [Hb] and Hct values for several species representing a range of diving and flying abilities. The values are highest for those species that both fly and dive, illustrating the duality of adaptive function of those parameters. As seen in Table 5, the Red-throated Loon (Gavia stellata) has the highest [Hb] and a very high Hct.

Table 5. Blood parameters of various species of aquatic and nonaquatic birds.

Species	[Hb] (g/100ml)	Hct (%)	Source
domestic hen	10.4	34.0	Bond & Gilbert, 1958
pigeon	19.4	52.0	Bond & Gilbert, 1958
South American Hummingbird	18.1	62.1	Johansen et. al., 1987
Red-throated Loon	20.7	54.0	Bond & Gilbert, 1958
Adelie Penguin adult	16.5	46.2	Milsom et. al., 1973
Adelie Penguin chick	11.1	29.0	Milsom et. al., 1973
Gentoo Penguin adult	16.4	43.4	Milsom et. al., 1973
Gentoo Penguin chick	11.8	31.1	Milsom et. al., 1973

It has the ability to dive to 60m for up to 15 minutes and also sustain flight for long migratory distances (Bond and Gilbert, 1958). The adult Common Murre has a relatively high [Hb] and Hct. These elevated blood parameters, along with a high blood buffering capacity (Lenfant, et. al., 1969), are physiological adaptations suitable to diving. High [Hb] and Hct, along with a low affinity for oxygen in murre blood (Lenfant, et. al., 1969) facilitates release of oxygen to the tissues and is adaptive for sustained aerial flight.

Although the oxygen carrying capacity of the blood contributes to both aerial and aquatic flying abilities, the air sac respiratory system comprises a substantial reservoir of oxygen (Butler and Jones, 1982). The Common Murre dives upon inspiration, fully expanding its air sacs before submerging (personal observation). The relatively large volume of the respiratory system of birds provides a usable oxygen store at least 1.5 times as large as that of the blood (Butler and Jones, 1982). This air sac oxygen store, along with the relatively high oxygen carrying capacity of the blood, enhances the murre's ability for aerial and possibly aquatic flight.

Myoglobin

Most vertebrates exhibit three different muscle fiber types - white, red, and intermediate. The color is largely due to the amount of myoglobin within the tissue (Hochachka and Somero, 1984). These white, red and intermediate fibers distinctly differ in their physiological characteristics and allow partitioning of intramuscular metabolic work (Hochachka and Somero, 1984; Hoppeler and Billeter, 1991). White muscle fibers have low myoglobin concentrations, few mitochondria, low respiration rate, high glycolytic rate and high buffer levels (Hochachka and Somero, 1984). Red muscle fibers have high myoglobin concentrations, abundant mitochondria, high respiration rate, low glycolytic rate and low buffer levels (Hochachka and Somero, 1984). Intermediate fibers have characteristics of both white and red (Hochachka and Somero, 1984). Animals with high myoglobin concentrations in their muscle are therefore likely to have abundant mitochondria and rely primarily on aerobic metabolism. Early investigations showed that the "nearly black" muscle tissue in Sperm Whales (Physeter catodon) was due to its exceptionally high myoglobin, and the correspondingly high oxygen store enabled prolonged, deep dives (Scholander, 1940). Since muscle myoglobin can

serve as both a means of facilitating rapid oxygen transfer from the blood to the muscle cell mitochondria and as an oxygen store, capacity for sustained aerial and/or aquatic flight has been attributed to elevated myoglobin concentration in the pectoralis muscle in birds (Wittenberg, et. al., 1975; Butler and Jones, 1982; Pages and Planas, 1983; Hoppeler and Billeter, 1991). Pigeons and hummingbirds, both strong, sustained aerial fliers, show very high myoglobin concentrations in the pectoralis as compared to more terrestrial, intermittant flying birds, such as the pheasant or grouse (Pages and Planas, 1983; Johansen, et. al., 1987; Davis and Guderley, 1990). Similarly, in a study by Davis and Guderley (1987), the Atlantic Puffin (Fratercula arctica), a seabird that routinely dives to 68m, showed a pectoralis Mb concentration that was 26-fold higher than in the pheasant and 1.8-fold higher than in the pigeon. However, though myoglobin concentration may be higher in both strong aerial and aquatic fliers, the function of that myoglobin may be different between these groups. Myoglobin in the pectoralis of strong aerial fliers, such as the pigeon, is thought to be more important in facilitating oxygen transfer from the capillaries to the mitochondria than as an oxygen store (Weber, et. al., 1974; Cole, 1983). In birds specialized only for diving, such as the penguin, where Mb concentration in the pectoralis is 5.8-fold higher than in pigeons and 4-

fold higher than in puffins, myoglobin may be more important as an oxygen store (Weber, et. al., 1974). As the Common Murre is both an aerial and aquatic flier, its pectoralis muscle Mb would probably serve two purposes; that of an oxygen reservoir while diving and of oxygen delivery to the tissues while exercising (flying). Although differences in techniques used to determine myoglobin concentration at this time precludes cross species comparisons with the Common Murre, the intensely dark red color of the adult murre pectoralis imples that its myoglobin concentrations are comparable to other diving birds such as the Common Loon, Tufted Puffin and Pigeon Guillemot, and marine mammals such as the harbor seal (personal observation).

Myoglobin intensity (representing concentration) of the pectoralis muscle increases significantly in the Common Murre chick as it matures (Figure 3; Appendix B, Figure B-1). This increase may be controlled by both genetic and environmental factors. While on the colony and relatively sedentary, an increase in muscle myoglobin may be due to genetically determined developmental processes. In fact, an increase in muscle myoglobin during the embryonic process in birds has been determined (Pages and Planas, 1983). Further development of the pectoralis muscle when the murre chick is at sea, however, may also involve environmental influences. Pattengale and Holloszy (1967) found that prolonged exercise, regularly performed, significantly increased the

concentration of myoglobin in skeletal muscle in the rat. Further, Cole (1983) determined that hypoxic conditions in mammals will stimulate the production of myoglobin. Thus, it is unclear whether the increase in myoglobin seen in the OP age group (approximately 37 days-at-sea old)(Figure 6) is due to ontogenetic myoglobin expression and/or increased muscle activity (e.g., practice flapping, practice diving) and low oxygen tensions during practice dives. In either case, when younger chicks must use their pectoralis for sudden activity such as a predator escape dive attempt, the low muscle myoglobin suggests glycolytic capacity would have to provide the metabolic needs of the pectoralis muscle. Independent murre chicks (IC) show a further increase in Mb intensity, approaching adult levels (Figure 6). This increase infers oxygen stores that are now sufficient for normal and extended foraging dives, consistent with the fact that independent chicks must forage for themselves. Peak myoglobin intensity is apparently not reached until the adult stage. Given that aquatic flight (diving) for the independent chick is necessary for immediate survival, and aerial flight probably is not, it is reasonable to assume that most of the independent chicks collected had not yet performed extensive aerial flight. The difference in myoglobin intensity between this group and the adults may then represent the additional amount of pectoralis myoglobin necessary for sustained aerial flight.

A similar pattern exists in the gastrocnemius muscle; myoglobin intensity increased significantly in murre chicks until finally attaining the adult levels (Figure 4). This increase does not become apparent until the chicks have reached the maturity of the OP age group. The lack of increase in myoglobin intensity in the YP over the HD group may indicate that, 1) there was no synthesis of myoglobin in response to exercise (surface swimming) in the gastrocnemius, indicating that chicks were genetically equipped with sufficient myoglobin before leaving the nest; or 2) such a synthesis response is delayed and would take longer to manifest itself than the ten day mean difference in age between the two younger groups.

The heart muscle shows a significant increase in Mb intensity with maturation (Figure 5), though age explains only about 25% of the variation in heart myoglobin. As heart muscle is almost exclusively aerobic (Hochachka and Somero, 1984), adequate myoglobin may be critical for proper function; the higher myoglobin concentration (relative to pectoralis and gastrocnemius) in the HD and YP groups illustrates its importance. The increase in myoglobin intensity in older chicks probably reflects incurred overall activity and heart rates at those ages. Again, this may be due to an exercise effect; Catlett, et. al. (1978) determined that in pigeons that were prevented from flying, myoglobin concentration decreased in the heart muscle. It

appeared from this study that activity stimulated myoglobin synthesis in the heart, while inactivity stimulated myoglobin degredation. Normally active pigeons maintained a relatively constant myoglobin level. It may be that continued exercise after a relatively inactive existence on the colony may stimulate myoglobin sythesis in the heart muscle of the Common Murre, contributing to increasing myoglobin intensity during maturation. Moreover, the initiation of diving practice may stimulate myoglobin synthesis to the extent that heart myoglobin in the OP age group reaches adult levels. Finally, though Common Murre chick's heart myoglobin levels begin relatively high and increase somewhat with maturation, they are apparently maintained below pectoralis and gastrocnemius levels in the adult. This is likely due to the differences in metabolic profiles of the muscle tissues. The heart muscle, relying almost exclusively on aerobic metabolism, plausibly has greater oxidative substrates and elevated key oxidative enzymes than do the skeletal muscles and may not require as high a myoglobin content.

Lactate Dehydrogenase

Enzyme Activity

In the muscle tissues of most vertebrates, the choice

of metabolic pathways used to generate muscle contraction depends on oxygen availability (Castellini, et. al., 1981; Butler and Jones, 1982; Hochachka and Somero, 1984). When oxygen is not limited and oxygen tensions are high, such as during submaximal exercise or during shorter dives, ATP is generated aerobically, through oxidative phosphorylation in the Krebs cycle (Butler and Jones, 1982; Hochachka and Somero, 1984). When oxygen tensions are low, such as during rapid bursts of muscle contraction, during peak exercise, or when environmental oxygen is unavailable, ATP is generated anaerobically through the glycolytic pathway (Butler and Jones, 1982; Hochachka and Somero, 1984). As Figure 33 illustrates, glycolysis is the first step in the generation of metabolic energy from glucose or glycogen (Mathews and van Holde, 1990). Oxygen availability will dictate whether NAD^+ is regenerated via pyruvate oxidation in the Krebs cycle or via anaerobic reduction by LDH (Mathews and van Holde, 1990). The regeneration of NAD^+ is vital for both aerobic and anaerobic glycolysis to continue (Mathews and van Holde, 1990).

Analyzing the kinetic properties of LDH may yield information on the metabolic capacities of different tissues. The Michaelis-Menton constant, Km, is the substrate concentration at which an enzyme is at half of its maximum velocity (Mathews and van Holde, 1990). It is often used in describing an enzyme's characteristics because the

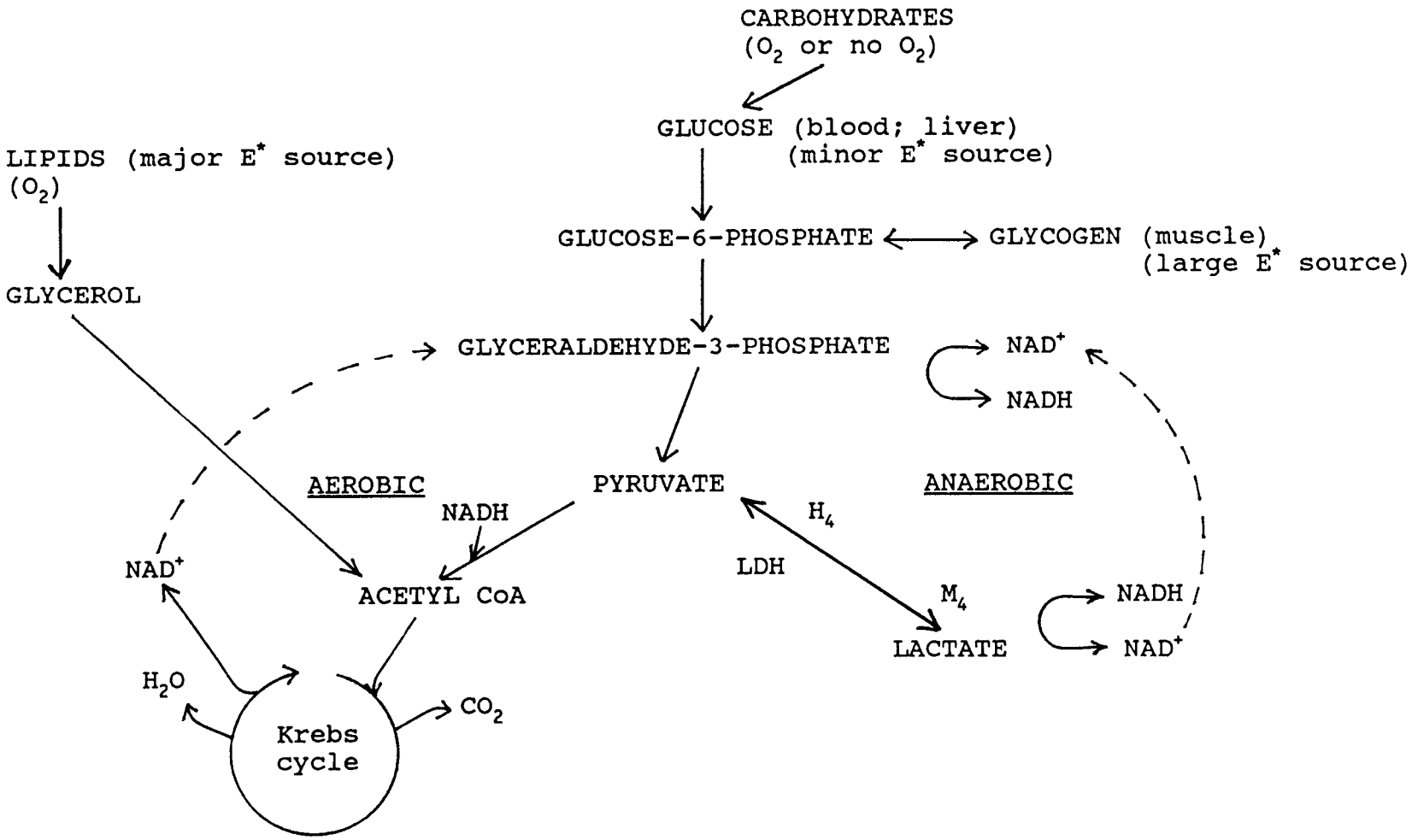


Figure 33. General metabolic map of aerobic and anerobic pathways of glycolysis.

*Energy.

greatest velocity change in a reaction occurs within a region of Km (Kuchel and Ralston, 1988). Km also reflects an enzyme's affinity for a substrate. A low Km for LDH indicates a high affinity for pyruvate which promotes its reduction to lactate, generating ATP anaerobically (Everse and Kaplan, 1975; Hochachka and Somero, 1984). A high Km for LDH indicates a low affinity for pyruvate, thus pyruvate is shunted into the Krebs cycle for oxidative phosphorylation, and ATP is generated aerobically (Everse and Kaplan, 1975; Hochachka and Somero, 1984).

The LDH Km regression analyses of pectoralis, gastrocnemius and heart muscle in Common Murre chicks, shows no significant correlation with maturation (Figures 10, 11, 12). As indicated by the r^2 values for all tissues, there is great variability in Km when estimated by age, and there was no discernible increase in Km with age of chick. Although there seems to be a general decrease in Common Murre chick pectoralis substrate affinities with maturation (Figure 10), age remains a poor predictor of Km. This great variability in Km may reflect the fact that assays were performed using a homogenate containing all five of the isozymes of LDH, each with an affinity expressed by the number of H and M subunits. Although a range of affinities might be advantageous in preserving LDH activity under varying oxygen conditions, one would expect to find an ontological pattern and/or specific tissue differences given

the likely predominance of one subunit type over another within a tissue sample. For example, the pectoralis muscle of the younger murre chicks may be best suited with a high affinity (low Km) for burst muscle use only, while older murres that use the pectoralis for diving (and practice diving) and eventually flying, may require a low affinity for pyruvate (high Km), assurring a preference of the muscle tissue for aerobic metabolism. Although no differences among age groups would be expected in the heart muscle, its affinities for pyruvate should be much lower than those of the pectoralis and gastrocnemius skeletal muscles, since its energy is derived primarily aerobically. Km values for the heart did not appear to differ from the pectoralis or gastrocnemius muscles (Figure 13; Table 3).

In the Common Murre it appears that in the three muscle tissues examined, all five possible LDH isozymes are usually expressed (Appendix B). The LDH subunits, H and M, are independently genetically regulated and show tissue specificity (Fersht, 1977). In variable environments, maintenance of genetic variability in LDH isozyme expression would seem valuable in assuring that optimal enzymatic function is viable within a physiological range. Given the lack of change in Km with maturation in the Common Murre, perhaps what is vital in the LDH catalyzed reaction is in regulating enzymatic sensitivity through varying the synthesis of these five LDH isozymes. Thus, a range of Km

is maintained to conserve metabolic function through the presence or absence of these isozymes, each with specific and optimal kinetic characteristics. Analysing kinetic parameters, such as Km, requires careful biochemical interpretation, since it represents affinities of all isozymes in addition to other cellular metabolites. Further, since the LDH reaction in this study was quantified by actually measuring the conversion of the cofactor, NADH, inappropriate concentrations of this important cofactor may have affected the kinetic results. The approach used in this investigation was based on the assumption that the activity of LDH under in vitro conditions may be considered as a relative measure of metabolic capacity in vivo. Thus, variability in LDH Km may indicate enzyme plasticity that would conserve enantiostasis (aerobically or anaerobically) in a range of biochemical environments, that may be encountered by the maturing Common Murre, although this postulation cannot be unequivocally stated.

Initial velocities of enzymes are often determined at increasing substrate concentrations to elucidate the profile of an enzyme's activity (Kuchel and Ralston, 1988). Small increases in substrate concentrations cause relatively large increases in reaction velocity, until the enzyme is saturated with substrate and its activity is no longer increased (Kuchel and Ralston, 1988; Mathews and van Holde, 1990). In the animal, physiological substrate concentrations

are similar to or lower than kinetically derived Km values (Fersht, 1977; Hochachka and Somero, 1984). In this study, the highest LDH Km value estimated from the direct linear plots was 0.48 mM. Therefore, of the five pyruvate concentrations used in the LDH activity analysis, 0.15mM and 0.30mM probably represent the physiological range of pyruvate concentrations in the Common Murre.

In the pectoralis muscle, there is a significant difference among age groups for activities at the 0.15mM and 0.30mM concentrations (Figure 7). The differences in LDH activities of the different age groups diminishes with increasing pyruvate concentration; differences among age groups do not exist at higher, physiologically unrealistic pyruvate concentrations, e.g., 1.5mM, 3.0mM, 15.0mM. This may be merely physical kinetic behavior of LDH in the presence of abnormally high pyruvate concentrations. It is interesting that age group differences are most evident at 0.15mM between the younger murre chicks (HD and YP groups) and the older birds, including adults. The lower LDH activities of the younger chicks is consistent with the fact that they do not use their pectoralis muscle except in escape dive attempts (personal observation). Therefore, though glycolytic capacity may be present, it is rarely needed by the younger chick. The higher LDH activity in the pectoralis of the older murres may be indicative of the increased anaerobic use of that muscle. Older chicks begin

to stimulate the pectoralis by wing flapping while on the water, and by submerging and attempting to follow the parent on foraging dives (personal observation). The highest LDH activity of the IC age group at 0.15mM may be evidence of increased diving and peak muscle stimulation in the physiological development of the pectoralis. Abandoned by the adult, the independent chick must now dive for its food and its lack of experience in underwater foraging probably increases the time invested in each foraging dive. However assuming that 0.15mM and 0.30mM is the physiological range for pyruvate concentration in the Common Murre, 0.30mM probably better represents an in vivo anaerobic pyruvate concentration and therefore a better point at which to interpret glycolytic capacity. The LDH activities at the lower pyruvate concentration, 0.15mM, may be more representative of LDH activities at more aerobic, sustained muscle activity levels.

The gastrocnemius muscle showed high variability within groups (Table 2), and no significant differences among age groups at any pyruvate concentration (Figure 8). The lack of differences among age groups in the physiological range is not surprising. Common Murre chicks, as well as adults, frequently use the gastrocnemius muscle to surface swim. As previously noted, chicks do not have time to gain ability through training; as soon as they leave the colony, they must sustain muscle activity to keep up with the parent.

The heart muscle also shows no differences among age groups in LDH activity at physiological pyruvate concentrations. One would expect a central organ such as the heart to function with equal efficiency in all age groups. The significant difference in LDH activity among age groups at the highest pyruvate concentration, 15.0mM, yields information of LDH kinetic behavior, but for in vitro conditions only. After exercise to exhaustion (i.e., sustained maximal exercise), canine skeletal muscle's peak pyruvate concentrations were only 0.8mM (Vesell and Pool, 1966). Therefore, LDH activity in the murre at 15.0mM is probably of no physiological importance.

Isozymes

The LDH activities in the maturing Common Murre indicate the capacity to modify function to accomodate the metabolic needs of the muscle tissue. Affinity values (Km) and activity profiles, however, only allude to the finer biochemical characteristics of the muscle. The proportions of the five possible LDH isozymes that can occur in a muscle tissue, H_4 (LDH 1), H_3M_1 (LDH 2), H_2M_2 (LDH 3), H_1M_3 (LDH 4) and M_4 (LDH 5), represent more accurately the oxidative and glycolytic capacities of a particular muscle type. Additionally, the differential association of these isozymes with specific classes of muscle tissues with unique

biochemical properties (red, intermediate, and white fiber types) provides flexibility in muscle contractile function (Hochachka and Somero, 1984). For example, the high glycolytic rate of white muscle is associated with a predominance of M type LDH and anaerobic capacity. Conversely, in red, oxidative skeletal fibers prevalent in aerobically metabolizing tissues, H type LDH predominates.

The pectoralis muscle of birds varies in the proportion of H and M LDH subunits corresponding to their modes of flight (Wilson, et.al., 1963; Dial, et. al., 1987). Wilson, et. al. (1963) found that birds capable of powerful, sustained flight, such as Wilson's petrel (Oceanites oceanicus) has only the M_1H_3 and H_4 isozymes in the pectoralis muscle. Birds that engage in sudden bursts of muscle activity for only short distances, such as the Ruffed Grouse (Bonasa umbellus) have predominantly M_4 in the pectoralis muscle. The Common Murre adult shows similar amounts of all five LDH isozymes in the pectoralis muscle (Figure 19), reflecting the plasticity needed in its pectoral function. Because the murre is capable of sustained flights of considerable speed, relatively high levels of H type LDH in the pectoralis facilitates aerobic metabolism by its low affinity for pyruvate, allowing pyruvate to be oxidized in the mitochondria. Its rich stores of myoglobin also promote continued aerobic metabolism while diving. Alternatively, the presence of the

M type LDH isozymes allow substantial rates of glycolysis to proceed during peak muscle work, or when oxygen stores are depleted during a prolonged dive. White muscle fibers rich in M LDH are also used mainly during take off and landing, and when birds undergo sudden changes in flight direction (Dial, et. al., 1987). Common Murres have high wing loading, requiring high power output at low speeds, such as during take off from the water. The M type LDH isozymes are likely recruited during these bouts of peak muscle work. On the whole, the adult Common Murre pectoralis muscle appears to be well adapted to a variety of power demands, supported by aerobic and anaerobic pathways as required.

Common Murre chicks undergo a change in pectoralis isozyme composition as they mature (Figure 19; Appendix B, Figure B-2). LDH 1, LDH 2, LDH 3, and LDH 4 increase with age, whereas LDH 5 does not (Figures 14-18). Ontological differentiation of isozyme patterns is variable among vertebrates. In the mouse, all embryonic tissues have a predominance of LDH 5 initially, the isozyme pattern gradually shifting to more aerobic LDH isozymes with development, until the adult pattern is finally reached (Markert and Ursprung, 1962). Due to the relatively hypoxic environment of a mammalian embryo, and because the mitochondria are few and immature, the initial dependence on anaerobic glycolysis is high (Hochachka and Somero, 1984). Markert and Ursprung (1962), also found that the major

differentiation of isozyme patterns in the mouse occurs during the neonatal and juvenile period (three weeks after birth). In the Japanese Lesser Horseshoe Bat the isozymes of the pectoralis muscle shift from predominantly LDH 2 and LDH 3 in the newborn stage to LDH 4 in the older flapping stage to predominantly LDH 1 and LDH 2 in adults (Yokoyama, et. al., 1979); again, an apparent shift to a more aerobic isozyme composition. In the flapping stage, LDH 4 is the dominant isozyme likely due to the burst muscular activity involved in flapping and exercising the wings in preparation for flight (Yokoyama, et. al., 1979). In the adult stage the increase in LDH 1 and LDH 2 indicates that the adult pectoralis is now poised for slow and sustained yet highly maneuverable flight (Yokoyama, et. al., 1974). The Common Murre chick, like the bat, shows a physiological shift in pectoralis isozyme composition during maturation related to its flying and diving activity. LDH 1 and LDH 2 are not prevalent in the younger HD and YP age groups (Figure 19), which reflects the lack of sustained use of this muscle for flying or diving. LDH 5 is the predominant isozyme in these younger chicks. Though the murre chick pectoralis muscle is rarely used in early life, the muscle is poised for short burst activity if the chick must attempt an escape dive to elude predators, such as gulls (personal observation). Throughout the age groups, LDH 5 is maintained at a constant level of intensity (Figure 19)

indicating the retention of anaerobic burst capability in the pectoralis throughout life. The profound changes in isozyme composition in the pectoralis with maturation are in the other four isozymes, particularly LDH 2 and LDH 3. Again, whether these increases in aerobic ability (increase in H subunits) in the pectoralis are genetically programmed or environmentally induced mechanisms is not distinguishable. With wing and muscle growth, use of the pectoralis increases as the older chick exercises it by more frequent dive attempts and wing flapping (pers. obs.). Training adaptation leads to alterations in isozyme composition (Hochachka and Somero, 1984). The largest adjustment in isozyme composition is with an increase in LDH 3. This isozyme probably increases the murre's capacity to alter metabolic pathways rapidly, since it is poised for both aerobic and anaerobic function (M_2H_2).

Although the regression analyses for the gastrocnemius muscle showed a significant increase in LDH 1, LDH 2, LDH 3, and LDH 4 with maturation, the r^2 values are low, with age explaining only 7-35% of the variability in intensity (Figures 20-24). Figure 25 illustrates the lack of remarkable increase in the five LDH isozymes (see also Appendix B, Figure B-2). This is consistant with the at-sea activity of the chick. Upon leaving the nesting colony, a murre chick is essentially thrust into a sustained gastrocnemius activity regime, constant submaximal surface

swimming necessary to accompany its parent. It appears that the chick leaves the colony with the development of its gastrocnemius muscle LDH isozyme composition complete.

Because not all LDH isozymes were present in the heart of all chicks, isozyme composition was analysed in two ways. First, when the isozyme was present, its intensity (concentration) was measured and regressed with age of chick. The second analysis looked for differences among age groups on the frequency of isozyme occurrence. For chicks possessing LDH 1, LDH 2, LDH 3, LDH 4, or LDH 5, the heart muscle shows no significant change in those isozyme intensities with maturation (Figures 26-30). Additionally, LDH 1 is by far the dominant heart isozyme throughout all age groups (Figure 31; Appendix B, Figure B-2). The dominance of this aerobic isozyme makes intuitive sense given that at normal, submaximal levels, the heart derives most of its energy (65%) from the oxidation of fatty acids and 35% from the oxidation of glucose in aerobic respiration (Everse and Kaplan, 1975). However, during prolonged, peak exercise, the heart will utilize the oxidation of lactate for as much as 60% of its energy output (Everse and Kaplan, 1975; Hochachka and Somero, 1984).

Though there was no change in isozyme intensity with age (when the isozyme did occur), the X^2 analysis did reveal a change in the frequency of occurrence of some isozymes. The younger chicks in the HD and YP age groups showed a more

frequent occurrence of LDH 2 and LDH 3, and particularly the most anaerobic isozymes, LDH 4 and LDH 5 (Figure 32). The heart, although fundamentally an aerobic muscle, has the capacity to reduce pyruvate to lactate under severely limited oxygen conditions (Hochachka and Somero, 1984). Thus, the murre has this emergency capacity if a dive is prolonged and oxygen supply to the heart becomes dangerously low. This seems a reasonable arrangement since the young chick may encounter frequent periods of anoxic stress when seas are rough and keeping up with its parent is strenuous. As the chick matures the decrease in occurrence of anaerobic isozymes may reflect the increase observed in skeletal (or heart) muscle oxygen stores (Mb) thus reducing the need for anaerobic capacity.

This decrease in occurrence of LDH 2, LDH 3, LDH 4, and LDH 5 from chick to adult is interrupted by an increase in occurrence of all those isozymes in the independent chicks. Studies conducted by Lindy and Rajasalmi (1966) and Dawson, et. al. (1964), revealed that ambient oxygen tensions suppress or induce synthesis of LDH subunits. Incubation of a chick embryo in an hypoxic environment resulted in an increase in the proportion of M subunits, whereas incubation in 100% oxygen supressed the synthesis of M subunits (Lindy and Rajasalmi, 1966). The independent murre chick, likely pressured to dive often while perfecting its foraging skills, would encounter more frequent periods of anoxia

associated with prolonged diving than would the older chicks still under parental care and not pressured to dive. Thus, the increased exposure of the heart muscle to transient periods of low oxygen tensions may stimulate sythesis of M subunits, resulting in a higher occurrence of LDH 3, LDH 4, and LDH 5. A greater abundance of LDH 2 may also aid in aerobic efficiency. The Chi-square analysis results indicated that LDH 3 occurs with the same frequency among age groups. Although the Chi-square value for LDH 3 is just significant (X^2=6.6), it may be advantageous to synthesize LDH 3 over LDH 4 or LDH 5 when oxygen tensions are habitually low. This would give the murre the capacity to resort to anaerobic glycolysis effectively when oxygen supplies are severely limited, yet would maintain aerobic capacity even when blood lactate concentrations are relatively high, since the heart uses lactate as an oxidative substrate.

CHAPTER V

SUMMARY

Blood hemoglobin concentration and hematocrit increase as the Common Murre matures from chick to adult. This increase in blood oxygen stores is compatible with both aquatic and aerial flight, although comparable blood parameter values of nonaquatic, volant birds suggests that enhanced blood oxygen stores are not exclusively a diving adaptation.

Muscle myoglobin increases with maturation in the pectoralis, gastrocnemius, and heart of the Common Murre. A punctuated increase in myoglobin levels in all tissues from the YP to the OP age group is concomitant with an increase in activity, such as practice diving and flying, until adult levels are reached. Myoglobin likely serves to facilitate oxygen transfer to the tissue mitochondria supporting sustained aerial flight, and serves as a valuable oxygen store to prolong aquatic flight.

Lactate dehydrogenase activities differ among age groups in the pectoralis only, particularly between the younger chicks (HD and YP age groups) and older birds, at <u>in vivo</u>, physiological pyruvate concentrations. A higher

pectoralis LDH activity in the HD and YP age groups facilitates anaerobic glycolysis, a suitable pathway for a relatively inactive muscle with low Mb levels. That no differences exist in LDH activity among age groups in the gastrocnemius and heart is plausible, given that both of these muscles must sustain activities similar to those of adults throughout maturation at sea.

Lactate dehydrogenase affinities for pyruvate (Km), an indication of anaerobic capacity, show no change with maturation nor show expainable differences among age groups. Additionally, great variability exists in the Km data, which could possibly reflect a range of affinities for pyruvate in changing biochemical environments in which LDH is active. Analysis of kinetic parameters such as Km, however, requires careful biochemical interpretation, since the five possible LDH isozymes represent several rate constants within even a small tissue sample. Therefore, the lack of biological pattern in the Km values may be due to insensitive or inappropriate assay conditions.

LDH isozyme composition changes occur in the muscles of the Common Murre to facilitate changes in muscle metabolic requirements with maturation. In the murre chick pectoralis, the aerobic capacity is enhanced (increases in LDH 1, LDH 2, LDH 3) and anaerobic abilities are maintained (high and unchanging levels of LDH 5 with increase in LDH 4) during growth at sea. The gastrocnemius muscle maintains

aerobic and anaerobic capabilities throughout maturation (relatively constant, similar amount of all five isozymes). Although primarily an aerobic metabolizing muscle as reflected by high levels of LDH 1 throughout maturation, the heart may synthesize LDH 3, LDH 4 and/or LDH 5 for anaerobic capacity when oxygen is severely limited.

The LDH isozyme pattern of a muscle represents the metabolic framework of a given tissue. It is evident that the tissues examined in the Common Murre have a heterogeneous isozyme population; this enzymatic heterogeneity grants the murre several biochemical pathways to support the metabolic needs of muscle work. When the murre chick leaves the colony, myoglobin levels in the gastrocnemius are evidently adequate for sustained surface swimming, but are also supported by similar levels of all LDH isozymes. Thus, power output of the young murre chick gastrocnemius when surface swimming is well suited to match adult output, necessary for the chick's survival. The adult gastrocnemius retains the isozyme profile of the chick, yet has augmented myoglobin levels which likely facilitate muscle use when surface swimming and when aerial flight corrections are made (i.e., for use as rudders). Thus, the gastrocnemius muscle is poised for either glycolytic pathway, with myoglobin possibly influencing when they are used. The heart muscle displays the capacity for primarily aerobic metabolism throughout maturation (high levels of LDH 1), yet preserves

the ability to synthesize other, more anaerobic isozymes as a metabolic safety net when oxygen tensions are dangerously low. The younger chick's pectoralis muscle is poised primarily for burst activity (e.g., predator escape dives) as evident by a large amount of LDH 5 and little myoglobin or aerobic isozyme metabolic support. With maturation to adult age, LDH 5 maintains high levels to accomodate low oxygen tensions encountered in prolonged dives. Myoglobin and aerobic LDH isozyme levels increase to facilitate the murre chick's increased muscle activity as it matures at sea and the adult murre's muscle metabolic demands of aerial and aquatic flight.

In the Common Murre blood and muscle oxygen stores and LDH isozyme compostions clearly support both aerobic and anaerobic metabolism as required by cardiac and skeletal muscle activity in the adult bird. Moreover, the ontogenetic changes in these physiological traits, and the underlying metabolic organization, prepare the young murre chick fro the physiological demands it must meet under various environmental conditions as it matures into an actively flying, swimming and diving bird that lives out its life at sea.

APPENDIX A

AGE, AGE GROUP DESIGNATION, CULMEN LENGTH, WEIGHT AND SEX OF COMMON MURRES

Common Murre	Age (days at sea)	Age Group	Culmen (mm)	Weight (g)	Sex
WAW005C	2.1	HD	19.5	225	F
WAW002C	3.4	HD	20.0	283	F
WAW003C	3.4	HD	20.0	210	M
WAW006C	3.4	HD	20.0	286	F
WAW008C	3.4	HD	20.0	288	F
DHV072C	3.4	HD	20.0	240	M
DHV039C	4.7	HD	20.5	275	M
WAW001C	6.0	HD	21.0	304	F
WAW004C	6.0	HD	21.0	322	M
WAW012C	6.0	HD	21.0	209	F
WAW011C	7.3	HD	21.5	211	M
DHV103C	7.3	HD	21.5	358	M
DHV073C	8.6	HD	22.0	261	M
WAW007C	8.6	HD	22.0	262	F
WAW009C	10.0	HD	22.5	276	M
DHV071C	11.3	HD	23.0	383	F
WAW010C	12.6	HD	23.5	306	M
WAW013C	12.6	HD	23.5	260	F
WAW014C	17.8	HD	25.5	339	F
DHV070C	13.2	YP	23.7	475	F
DHV102C	20.0	YP	26.3	621	M
DHV104C	21.0	YP	26.7	592	M
DHV107C	25.7	YP	28.5	728	F
DHV108C	25.7	YP	28.5	602	M
DHV116C	27.1	YP	29.0	678	F
DHV115C	27.1	YP	29.0	586	F
DHV114C	29.7	YP	30.0	656	F
WAW020C	29.7	YP	30.0	660	F
DHV128C	37.6	OP	33.0	870	F
DHV131C	38.9	OP	33.5	708	F
DHV132C	39.4	OP	33.7	914	M
WAW021C	42.8	OP	35.0	867	M

Common Murre	Age (days at sea)	Age Group	Culmen (mm)	Weight (g)	Sex
WAW100C	57.8	OP	40.7	887	M
WAW025C	62.6	OP	42.5	915	M
WAW101C	67.3	OP	44.3	818	F
WAW026C	69.2	OP	45.0	973	M
WAW027C	82.3	OP	50.0	1034	M
WAW254IC	56.0	IC	40.0	883	F
WAW250IC	56.8	IC	40.3	723	F
WAW103IC	61.3	IC	42.0	822	M
WAW105IC	62.6	IC	42.5	839	M
WAW252IC	62.6	IC	42.5	946	M
WAW102IC	66.5	IC	44.0	783	F
WAW251IC	75.2	IC	47.3	875	-
WAW004A	-	AD	44.2	1068	M
DHV044A	-	AD	45.0	861	F
DHV108P	-	AD	46.0	1020	M
WAW106A	-	AD	46.0	955	M
DHV065A	-	AD	47.0	991	F
DHV103P	-	AD	47.0	886	M
DHV107P	-	AD	47.0	865	M
WAW020P	-	AD	47.0	951	M
WAW003A	-	AD	47.0	1077	M
DHV128P	-	AD	47.5	1054	M
DHV035A	-	AD	48.0	955	F
DHV117A	-	AD	48.0	928	F
DHV102P	-	AD	48.0	860	M
WAW021P	-	AD	48.0	1011	M
WAW002A	-	AD	48.8	1019	F
WAW253A	-	AD	49.0	950	F
WAW001A	-	AD	49.0	1033	M
DHV040A	-	AD	49.3	1073	F
WAW104A	-	AD	49.5	950	M
DHV036A	-	AD	50.0	955	M
DHV114P	-	AD	50.0	979	M
DHV116P	-	AD	50.0	970	M
DHV043A	-	AD	-	985	F
DHV132P	-	AD	-	985	M

APPENDIX B

POLYACRYLAMIDE GEL ELECTROPHORESIS OF MUSCLE MYOGLOBIN AND LACTATE DEHYDROGENASE ISOZYMES

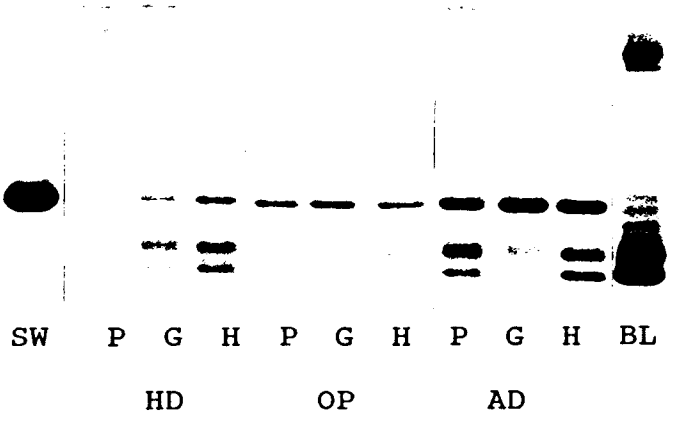


Figure B-1. SDS polyacrylamide gel electrophoresis of pectoralis (P), gastrocnemius (G) and heart (H) muscle myoglobin in the Common Murre, with Common Murre blood (BL) and Sperm Whale myoglobin (SW) references. Age groups represented: HD, youngest chicks under parental care; OP, older chicks under parental care; AD, adults.

Figure B-2. Polyacrylamide gel electrophoresis of pectoralis (PEC), gastrocnemius (GAS), and heart (HRT) muscle LDH isozymes. Age groups represented: HD, youngest chicks under parental care; OP, older chicks under parental care; AD, adults.

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