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Title: LDH activity and isozyme patterns in crayfish (*Orconectes nais*) under conditions of seasonal change.

Abstract Approved: Gaylen Hanfeld

In the crayfish *Orconectes nais*, LDH is present in two isozymatic forms. Isozymes from gill, abdominal muscle, heart and nerve have the same mobility indicating that they possess the same polypeptide composition. However, isozymes from hepatopancreas and intestine moving more cathodically suggests these isozymes are more positively charged. Isozyme patterns did not differ under different seasons suggesting no seasonal changes in the LDH isozyme composition of each tissue. The relative activities of the isozymes demonstrate that LDHs from gill, abdominal muscle, heart, and nerve are all M-type. These M-type LDHs with a ratio (0.33 mM pyruvate substrate/10 mM pyruvate substrate) close to 0.5 show that activity increases with an increase in pyruvate concentration. This is different from that of rat skeletal muscle which has a ratio of 1.67 indicating isozyme inhibition with high pyruvate concentrations. The effect of protease inhibitors on LDH activities from

hepatopancreas and intestine suggests that hepatopancreas has relatively low LDH activity. Gill, abdominal muscle, heart, and nerve show seasonality in the level of LDH activity. The physiological significance of the seasonality in LDH activity for seasonal adaptation is discussed.

Key words: Lactate dehydrogenase; LDH; *Orconectes nais*.

LDH ACTIVITY AND ISOZYME PATTERNS IN CRAYFISH *(ORCONECTES NAIS)* UNDER CONDITIONS OF SEASONAL CHANGE

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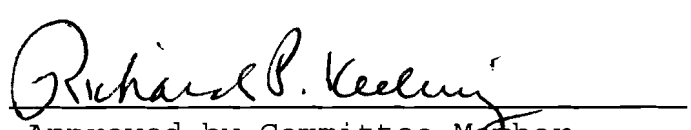

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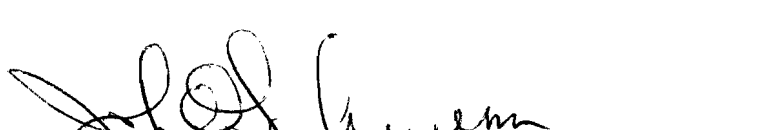

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PREFACE

This thesis was prepared following the publication style of *Comparative Biochemistry and Physiology*.

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Introduction

Lactate dehydrogenase has been shown to be a tetramer combining two polypeptides, M and H monomers, into five isozymes (Altman and Robin, 1969). The LDH-isozyme patterns are tissue specific (Markert and Ursprung, 1962). LDH-1 (the H_4 isozyme) is the most abundant of the five commonly encountered isozymes in the heart, kidney, and brain of vertebrates. LDH-5 (M_4) is the predominant form in liver and skeletal muscle (Vessel and Pool, 1966). Such tissue specificity implies that the isozymes have different physiological roles in tissue metabolism (Markert and Ursprung, 1962; Hornby et al., 1989). Everse and Kaplan (1975) proposed that polypeptide M catalyzes pyruvate reduction in anaerobic glycolysis while the function of polypeptide H is the oxidation of lactate in aerobic tissues. The other three hybrid isozymes have intermediate properties that vary with the ratio of their polypepetide types.

The properties of the LDH in crustaceans are much less studied. Also, the number of LDH isozymes varies in different crustacean species. In the snow crab, *Chionoecetes opolio*, LDH is present as five isozymes (Angers et al., 1994). In the shrimp *Palaemon serratas,* only two LDH isozymes have been demonstrated (Thebault and Bernicard, 1978). Only one LDH isozyme (M-type) has been reported in

crayfish (*Orconectes limosus*)(Ścislowski et al., 1982). However, this may be due to only heart and abdominal muscle having been investigated.

Crayfish exhibit considerable annual activity variation due to profound seasonal changes in temperature, day length, oxygen tension, and food supplies Personal observation). Furthermore, the molting cycle influences crustacean metabolism (O'Connor and Gilbert, 1968) and the breeding season may involve energy expenditures because of locomotory activity and gonadal development (Rice and Armitage, 1974). LDH is one of the enzymes taking part directly in carbohydrate metabolism and indirectly in the determination of the pathway for energy generation. LDH activity is, therefore, very important in studying the modalities of adaptation by an organism to environmental variation. This study focused on the effects of seasonal changes on tissue specificity and catalytic properties of LDH-isozymes in the crayfish (*Orconectes nais*) for the purpose of elucidating some of the mechanisms of seasonal acclimation.

Materials and Methods

Animals, tissues, and extraction of LDH

Crayfish, *Orconectes nais,* were obtained from Lake Wooster at Emporia State University during February (winter, approx. 5° C), May (spring, approx. 15° C), August (summer, 28° C), and November (fall, approx. 5° C) of 1996. In each season, a total of 6 male crayfish (>25g) were dissected immediately after capture. Extracts of each of the following tissues were prepared: gill, heart, nerve, abdominal muscle, hepatopancreas and intestine.

Extracts were prepared for electrophoresis by homogenizing the tissues in 2:1 ratio (V:W) of cold 0.01 M Tris-HCl, pH 8.0, containing 30% sucrose, 0.02 M EDTA and 0.05 M dithiothreitol. The homogenates were centrifuged at 4° C and 22,000 xg for 60 min and the resulting supernatant used for lactate dehydrogenase analyses.

Electrophoretic analysis

The LDH-isozymes were separated by agarose electrophoresis in a Tris-0.2 M glycine, pH 9.2 running buffer for 70 min at 170 volts. Gels were stained for LDH activity with a reaction mixture containing 0.05% nitroblue tetrazolium, 0.05% phenazine methosulfate, 0.05% NAD^+, 1% lactic acid and 0.2 M Tris-HCL buffer, pH 8.0. Rat heart was used as a reference.

Determination of LDH activity and relative activity

LDH activity was determined by assaying for both the direct (reduction of pyruvate to lactate) and reverse (oxidation of lactate to pyruvate) reaction as described by Narita and Horiuchi (1979). LDH specific activities from gill and abdominal muscle were expressed as the mean $\pm$ SE which was calculated from six individuals. The activity in the tissues of each animal was measured three times. Activities from pooled individual tissues (heart, nerve, hepatopancreas, intestine) were expressed as the mean of five assays. Relative activities were calculated from the ratio of LDH activities from pooled samples using 0.33 mM and 10 mM pyruvate as substrate. LDH activity for each tissue at each pyruvate concentration was determined three times. The relative activities of rat heart and rat skeletal muscle were calculated by the same methods used for those of crayfish tissues.

Effect of protease inhibitor on the LDH activity

Hepatopancreas and intestine tissues were homogenized in a buffer containing a protease inhibitor cocktail (ICN). It contained the following protease inhibitors per 100 ml homogenizing buffer: 40 mg AEBSF ([4-(2-Aminoethyl)-benzenesulfonyl fluoride]),

500 mg EDTA-Na_2, 0.1 mg Leupeptin, and 0.1 mg Pepstatin. LDH activity was measured as described above.

Protein determination

Protein concentration was determined with a BioRad assay (Bradford, 1976). Bovine serum albumin was the standard.

Statistics

Comparison of LDH specific activities from gills and abdominal muscle under different seasonal conditions were tested using a one-way analysis of variance (ANOVA) followed by a Student-Newman-Keuls test. Statistics were not run on the samples from heart, nerve, hepatopancreas and intestine since these represented pooled samples.

Results

Electrophoretic patterns

Figs. 1-4 show the electrophoretic patterns of LDH isozyme for animals tested during different seasons. All the tissues showed a single band. The LDH isozyme bands from gill, abdominal muscle, heart, and nerve had the same migration rate and ended at a position between the LDH-2 and LDH-3 isozymes from rat heart. Those from hepatopancreas and intestine had the same mobility and migrated more cathodically. Seasonal change showed no effect on the mobility and the number of isozymes in the tissues.

Reaction ratio for LDH

The ratios of LDH activity at 0.33 mM and 10 mM pyruvate as substrate from different tissues are listed in Table 1. The ratio is usually higher than 2 for the enzyme from tissues containing a preponderance of the H subunits and below 2 for the enzyme with a preponderance of M subunits (Ścislowski et al., 1982). The ratios of the extracts from the gills, abdominal muscle, heart, and nerve were all close to 0.5. The LDH isozymes of all these tissues, therefore, are all M-type.

Fig. 1 Photograph of the electrophoresis of LDH isozymes of different tissues from spring acclimated *O. nais*. 1: Rat heart (reference); 2: Gill; 3: Hepatopancreas; 4: Abdominal muscle; 5: Heart; 6: Intestine; 7: Nerve.

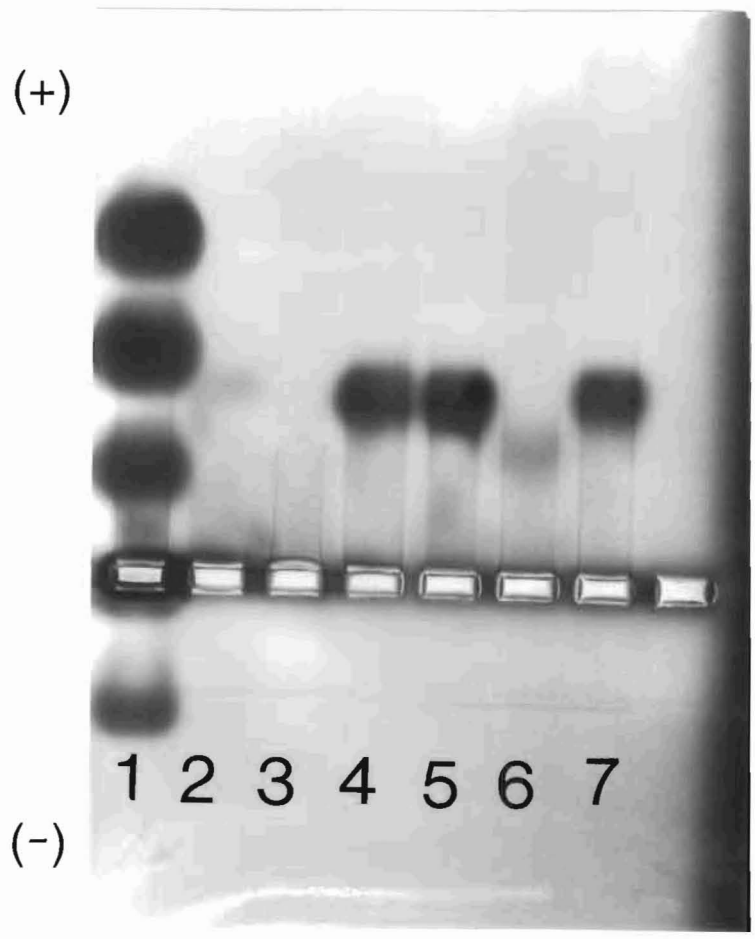
(+)
1 2 3 4 5 6 7
(-)

Fig. 2 Photograph of the electrophoresis of LDH isozymes of different tissues from summer acclimated *O. nais*. 1: Rat heart (reference); 2: Gill; 3: Hepatopancreas; 4: Abdominal muscle; 5: Heart; 6: Intestine; 7: Nerve.

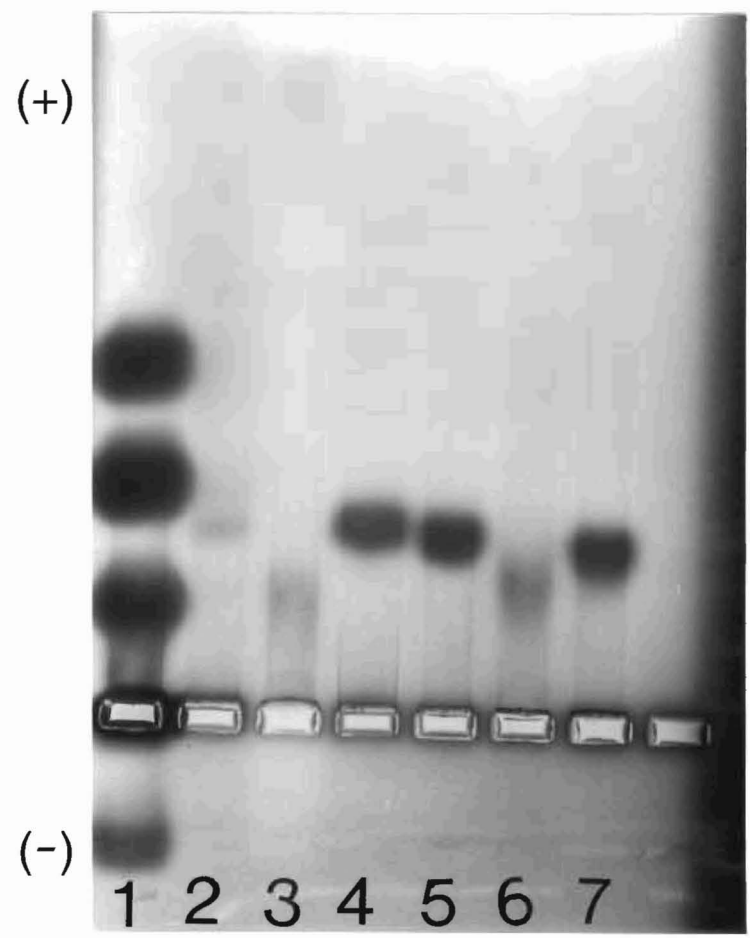
(+)
(-)
1 2 3 4 5 6 7

Fig. 3 Photograph of the electrophoresis of LDH isozymes of different tissues from fall acclimated *O. nais*. 1: Rat heart (reference); 2: Gill; 3: Hepatopancreas; 4: Abdominal muscle; 5: Heart; 6: Intestine; 7: Nerve.

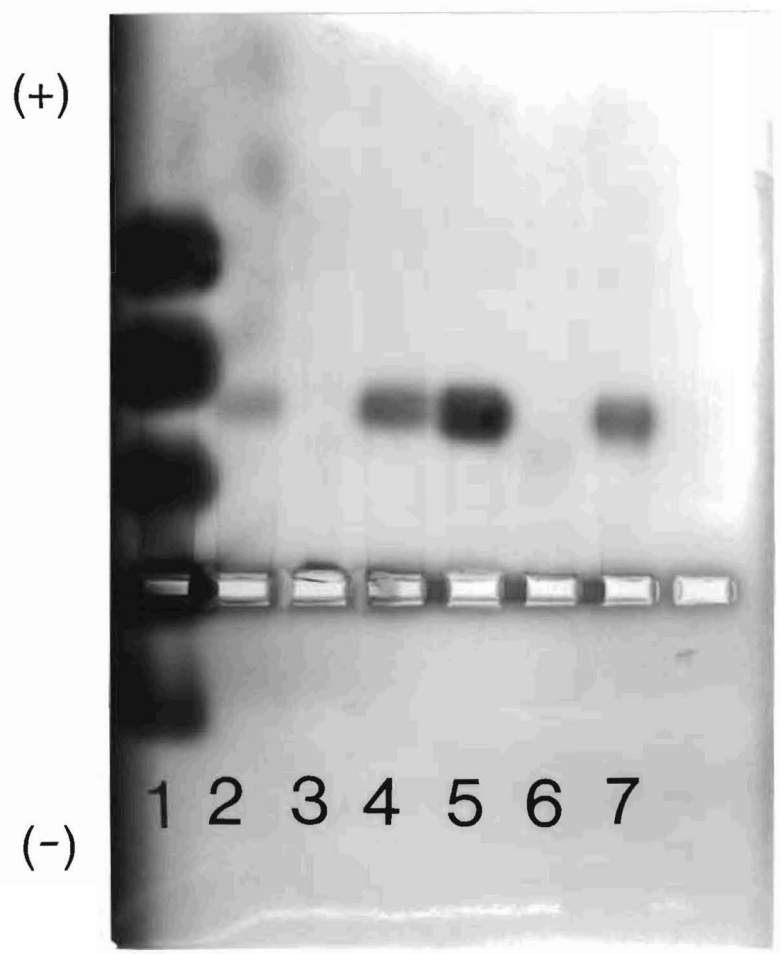
(+)
1 2 3 4 5 6 7
(-)

Fig. 4 Photograph of the electrophoresis of LDH isozymes of different tissues from winter acclimated *O. nais*. 1: Rat heart (reference); 2: Gill; 3: Hepatopancreas; 4: Abdominal muscle; 5: Heart; 6: Intestine; 7: Nerve.

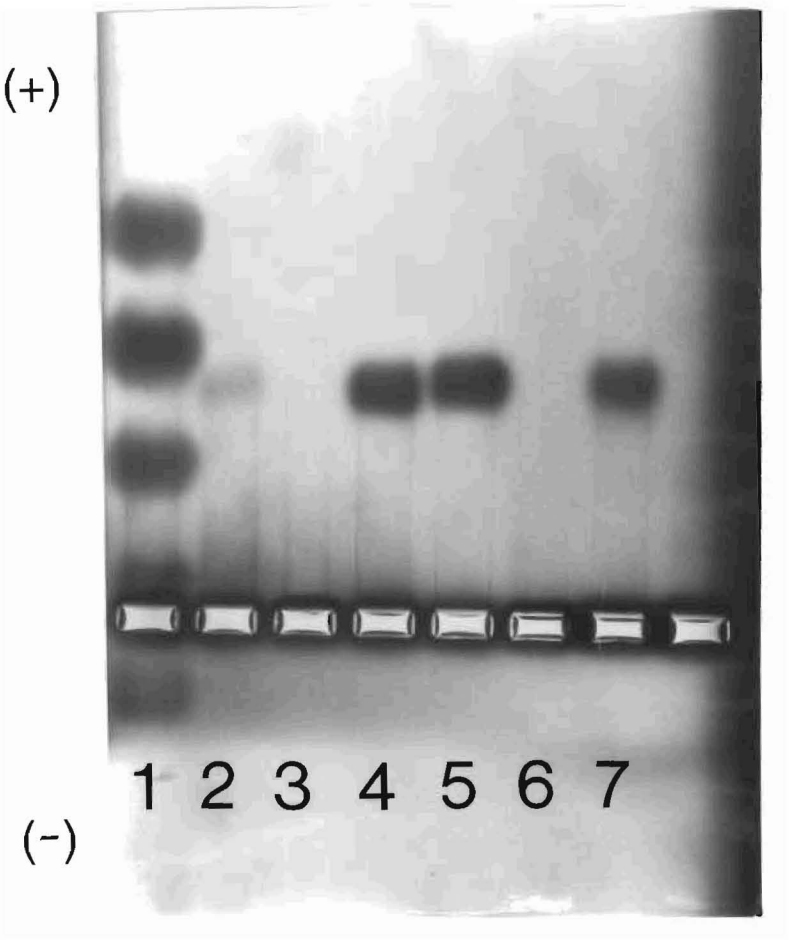
(+)
1 2 3 4 5 6 7
(-)

Table 1. Comparison of relative activities of LDH in fresh extracts of rat and crayfish (*O. nais*).

Tissue[a]	Ratio[b]
Rat heart	2.93
Rat skeletal muscle	1.67
Crayfish heart	0.50
Crayfish abdominal muscle	0.45
Crayfish gill	0.48
Crayfish nerve	0.66

[a] Individual pooled tissues are from fall acclimated *O. nais*.

[b] Relative activity is defined as the ratio of LDH activity using 0.33 mM and 10 mM pyruvate as substrate (0.33 mM/10 mM).

LDH specific activities with seasonal acclimation

The LDH activities of the crayfish gills (Fig. 5) from each season were relatively low compared to those of abdominal muscle, heart, and nerve (Figs. 6-8). However, the LDH activities of fall acclimated and winter acclimated animals were significantly higher than those of spring acclimated and summer acclimated animals ($F=36.7$, $p=0$) (Fig. 5).

In abdominal muscle, summer acclimated animals had the highest LDH activity (Fig. 6). The activities from fall tissues were significantly higher than those from spring tissues and winter tissues ($F=50.01$, $P=0$). Crayfish heart had a relatively high LDH activity in fall, the activity decreased to the lowest level in winter (Fig. 7). *O. nais* nerve tissue also possessed seasonality in LDH activity which approached the highest level in fall acclimated tissues (Fig. 8). LDH activities of hepatopancreas and intestine were relatively low in each season. All of the reverse reactions were undetectable except that of fall acclimated hepatopancreas (Table 2).

The effect of protease inhibitors on LDH specific activity

The use of protease inhibitors in the intestine extract increased the LDH activities for pyruvate reduction from 1.05 $\mu mol\ min^{-1}$ mg $protein^{-1}$ to

Fig. 5 LDH specific activities of gill extract from *O. nais* under different seasonal conditions. Direct reactions are expressed as µmole NADH oxidized min^{-1} mg $protein^{-1}$. Reverse reactions are expressed as µmole NADH produced min^{-1} mg $protein^{-1}$. Like letters for a given reaction are not significantly different (P=0).

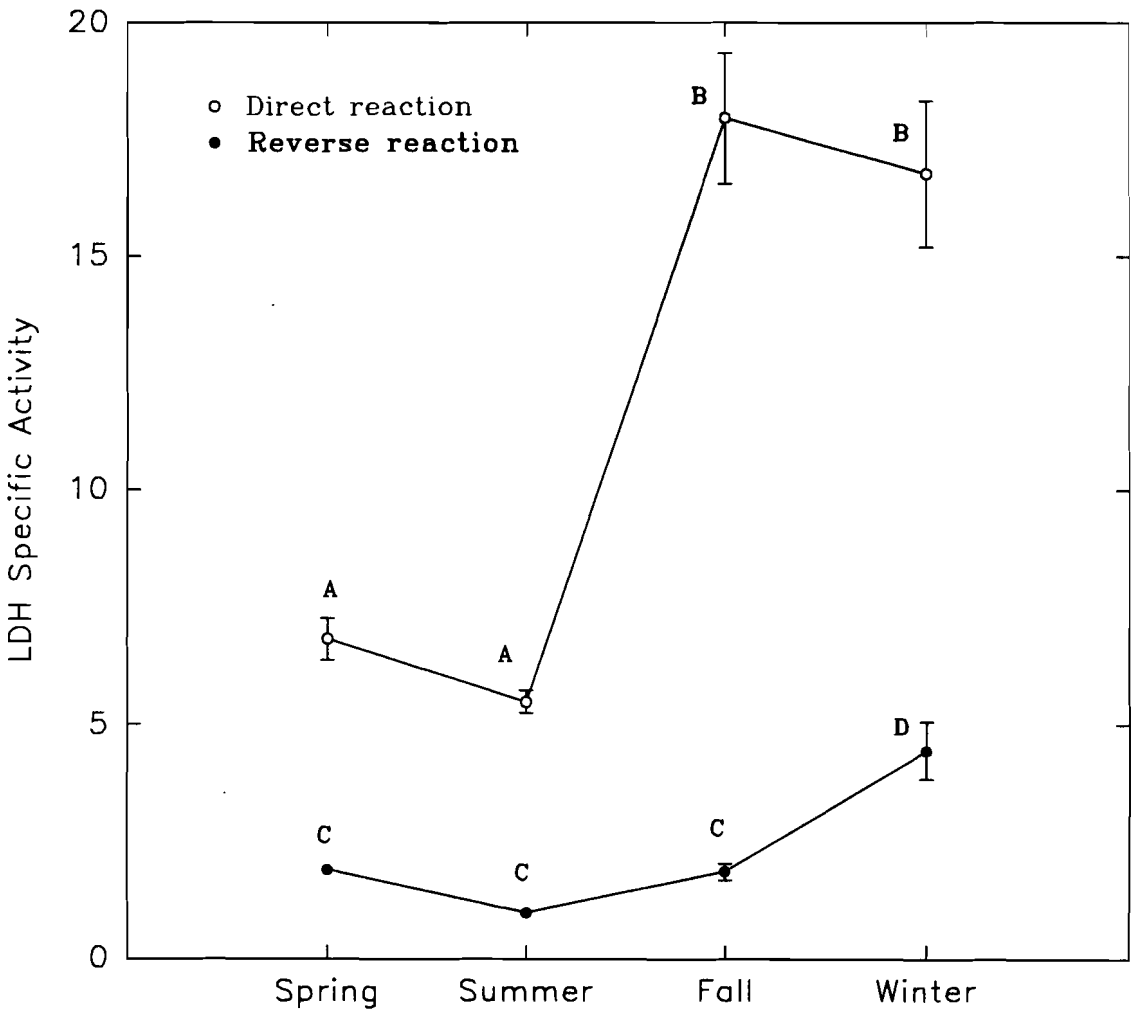
20
15
10
5
0
LDH Specific Activity
Direct reaction
Reverse reaction
A
A
B
B
C
C
C
D
Spring
Summer
Fall
Winter

Fig. 6 LDH specific activities of abdominal muscle extract from *O. nais* under different seasonal conditions. LDH specific activities are expressed as those in Fig. 5. Like letters for a given reaction are not significantly different (P=0).

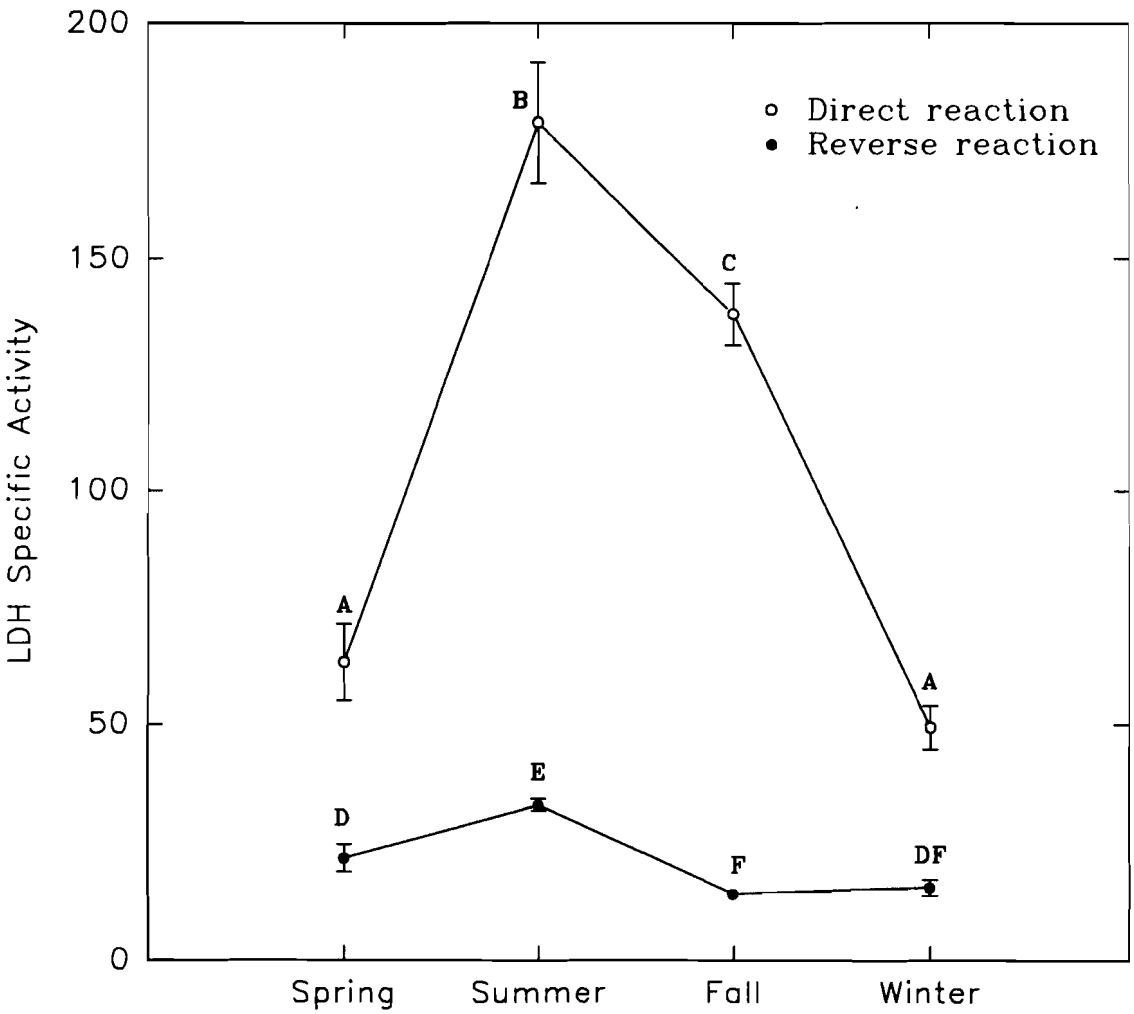
200
150
100
50
0
LDH Specific Activity
Direct reaction
Reverse reaction
A
B
C
A
D
E
F
DF
Spring
Summer
Fall
Winter

Fig. 7 LDH specific activities of pooled heart extract from *O. nais* under different seasonal conditions. LDH specific activities are expressed as those in Fig. 5.

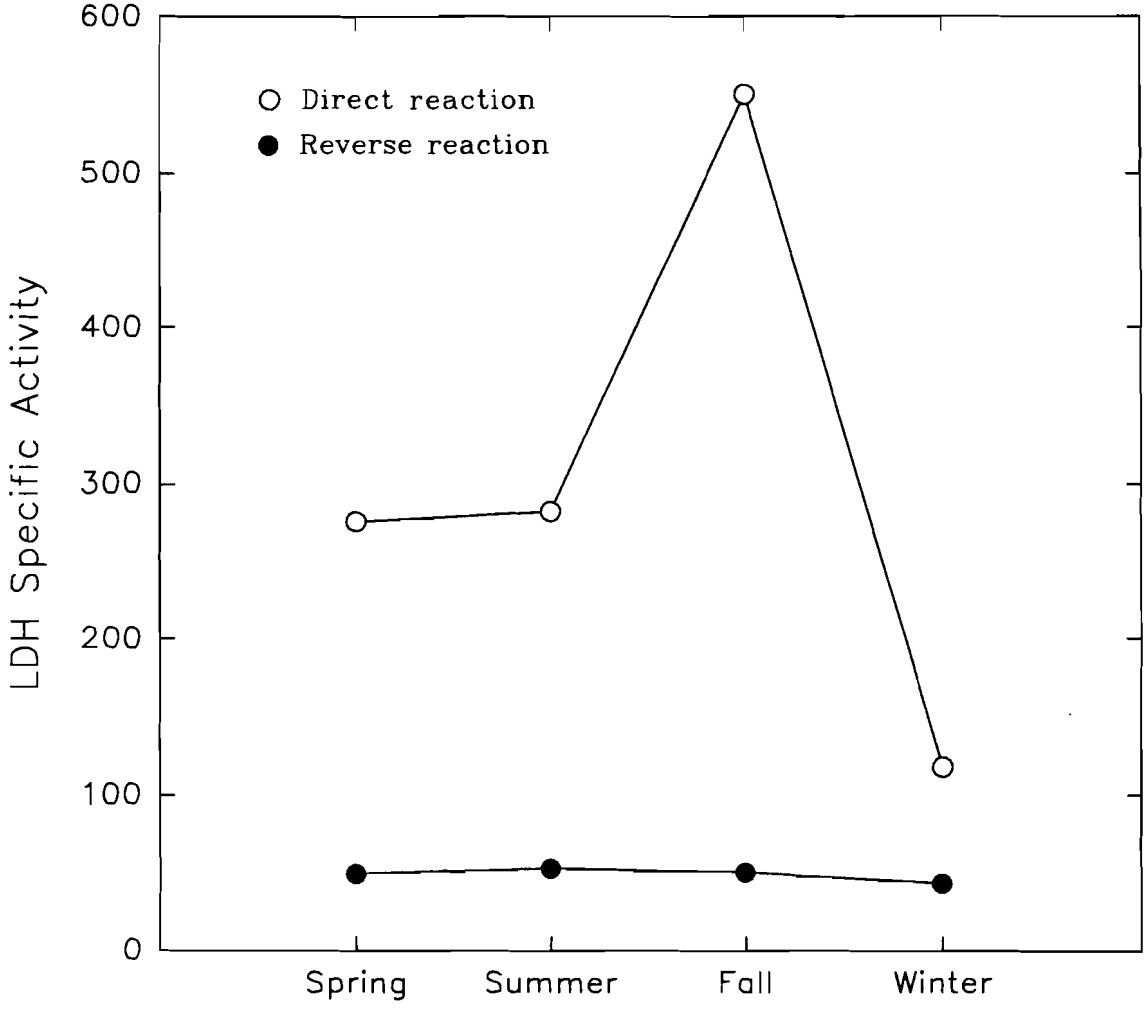
600
500
400
300
200
100
0
LDH Specific Activity
Direct reaction
Reverse reaction
Spring
Summer
Fall
Winter

Fig. 8 LDH specific activities of pooled nerve extract from *O. nais* under different seasonal conditions. LDH specific activities are expressed as those in Fig. 5.

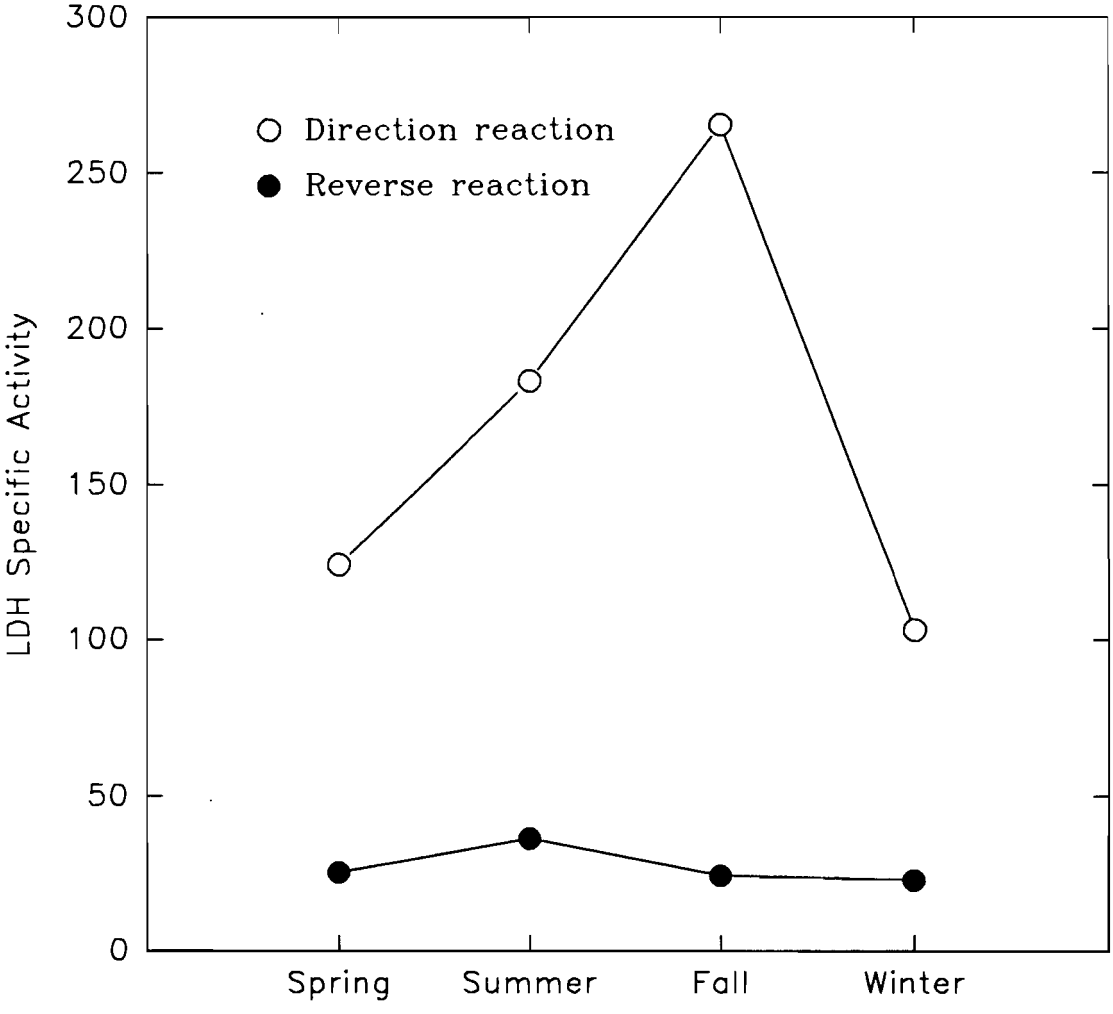
300
250
200
150
100
50
0
LDH Specific Activity
Direction reaction
Reverse reaction
Spring
Summer
Fall
Winter

Table 2. LDH specific activities of pooled hepatopancreas and pooled intestine from *O. nais* under different seasonal conditions.

Season	Hepatopancreas		Intestine	
	D. R.	R. R.	D. R.	R. R.
Spring	1.45	0.00	0.52	0.00
Summer	1.90	0.00	0.34	0.00
Fall	0.59	0.09	1.05	0.00
Winter	1.49	0.00	1.89	0.00

D. R. represents direct reaction. R. R. represents reverse reaction. Direct reactions are expressed as µmole NADH oxidized min^{-1} mg $protein^{-1}$. Reverse reactions are expressed as µmole NADH produced min^{-1} mg $protein^{-1}$.

112.11 μmol min^{-1} mg $protein^{-1}$ and the activities for lactate oxidation from undetectable to 9.71 μmol min^{-1} mg $protein^{-1}$ (Table 3). The effect of protease inhibitors on hepatopancreas LDH activity was not so dramatic. It increased the activity of direct reaction from 0.61 μmol min^{-1} mg $protein^{-1}$ to 3.47 μmol min^{-1} mg $protein^{-1}$, and the activity of reverse reaction from 0.10 μmol min^{-1} mg $protein^{-1}$ to 0.32 μmol min^{-1} mg $protein^{-1}$.

Table 3. The effect of protease inhibitor on LDH specific activities of fresh extracts from pooled hepatopancreas and pooled intestine from *O. nais*.

Tissue	Direct reaction		Reverse reaction	
	Without Inhibitor	With Inhibitor	Without Inhibitor	With Inhibitor
Hepatopancreas	0.61	3.47	0.10	0.32
Intestine	1.05	112.11	0.00	9.71

LDH specific activities are expressed as those in Table 2.

Discussion

Abdominal muscle, heart, gill and nerve tissue from *O. nais* possessed a single LDH isozyme band all with the same mobility. Ścislowski et al. (1982) also reported one band from abdominal muscle and heart in *O. limosus*. However, in the hepatopancreas and intestine from *O. nais*, the single band migrated more slowly. These observations indicate that the isozymes from gill, abdominal muscle, heart, and nerve, which migrated more rapidly toward the anode, are more negatively charged than those from the hepatopancreas and intestine.

There must be at least two different types of polypeptides in the present study which have different charges giving these two isozymes different mobilities. In general, L-specific lactate dehydrogenase is tetrameric, whereas D-specific LDH isozyme is a dimer. It is reported that nearly all the crustaceans have L-lactate dehydrogenase (Everse and Kaplan, 1973). Furthermore, the lobster, *Homarus americanus* shows the typical tetrameric L-specific LDH-isozyme pattern of vertebrates, and the crayfish, *O. limosus*, contains only one tetrameric L-lactate dehydrogenase (Gäde and Grieshaber, 1986). It has been shown that LDH from *Lampetra planeri* tissues exhibits only a single band containing polypeptides of a single type (Dell'Agata et al., 1988). It was proposed that polypeptide M catalyzes the pyruvate reduction in anaerobic glycolysis while the function of polypeptide H is the oxidation of

lactate in aerobic tissues (Everse and Kalpan, 1975). LDHs of each tissue except hepatopancreas catalyzed both the direct and reverse reactions. Therefore, it is impossible for these isozymes to be monomers such as those in *L. planeri,* as they would not be able to catalyze both reactions. It is also impossible for these isozymes to be dimers; they would not possess different charges because they would have the same composition.

There are several advantages for the LDH to exist in a polymeric structure. These include protecting the enzyme against inactivation by outside agents or in having interaction between subunits as a regulatory mechanism such as in lobster tail LDH (Everse and Kalpan, 1975). Based on this evidence, it is possible that LDH isozymes in *O. nais* are tetramers. This question is worthy of further investigation.

Isozyme patterns from *O. nais* seasons revealed no seasonal differences. The isozyme patterns of hepatopancreas and intestine were always different from those from gill, abdominal muscle, heart, and nerve. This suggests that LDH isozymes in *O. nais* are tissue-specific. This differs from the crayfish *O. limosus* which possesses one LDH isozyme without tissue specificity (Gäde and Grieshaber, 1986). Futher, isozyme patterns do not differ with season. This suggests there are no seasonal changes in the LDH isozyme composition in *O. nais* tissues. However, LDH specific activities in both the direct reaction

(F=50.01, P=0 in abdominal muscle; F=36.70, P=0 in gill) and reverse reaction (F=22.45, P=0 in abdominal muscle; F=21.46, P=0 in gill) revealed seasonality. This change appear to be due to a change in enzyme kinetics rather than alteration in isozyme composition.

The ratio of LDH activity at 0.33 mM and 10 mM pyruvate as substrate has been used to determine the relative proportion of M and H subunits in tissue extracts. A ratio greater than 2 represents an enzyme with a preponderance of H subunit; below 2 indicates a greater proportion of M subunit (Ścislowski et al., 1982). The ratios for all crayfish tissues tested in this study were around 0.5 (Table 1) so that the LDH can be qualified as the M-type. LDH in *O. nais* catalyzed both pyruvate reduction and lactate oxidation (Figs. 5-8). Kinetically, LDHs of crayfish tissues appear, therefore, to be hybrids of pure muscle type and pure heart type isozyme but with a dominant activity of pyruvate reduction.

The LDH from rat skeletal muscle had a reaction ratio of 1.67 which indicated an inhibition by high pyruvate concentrations. Crayfish abdominal muscle, however, displayed no substrate inhibition and the activity of this M-type LDH increased with the increase of pyruvate concentration over the range examined as indicated by the ratio of 0.5. My results showed that rat heart LDH had a high reaction ratio of 2.93. This is consistent with vertebrate heart LDH which has been shown to be much more

sensitive to the inhibition of high pyruvate concentrations than muscle type LDH (Everse and Kaplan, 1973). However, crayfish heart LDH is similar to abdominal muscle LDH which showed no inhibition to high pyruvate concentrations, but increased its activity with the increase of pyruvate concentrations. Everse and Kaplan (1973) hypothesized that M type isozymes predominate in anaerobic tissues such as skeletal muscles while H type isozymes occurs only in aerobic tissues such as the heart and brain. This hypothesis appears not to be applicable to the crayfish *O. nais* whose type M LDH predominates not only in abdominal muscle but also in heart and nerve.

Several studies have demonstrated the existence of seasonal changes in crustacean metabolism (Rice and Armitage, 1974; Dendinger and Schatzlein, 1973). Many of these seem to be associated with thermal acclimation (Rice and Armitage, 1974). In general, biological reactions respond to an increase in temperature by an increase of rate and decrease their rate if the temperature is lowered (Kerkut and Taylor, 1956). Comparison of LDH activities of nerve, heart, and abdominal muscle from winter acclimated animals and those from summer acclimated ones in the present study showed that the activities from summer tissues are higher. Popham and Dandy (1976), however, reported an increase in the LDH specific activity in crayfish *C. bartoni* abdominal muscle following cold acclimation. The activity of *O. nais* abdominal muscle, however, revealed an opposite

result. In addition, nerve tissue and heart muscle also had lowest LDH activities in winter.

Natural acclimation is a complex process. Environmental fluctuations associated with seasonal changes are the major factors facilitating adjustments of aquatic organisms. Cyclic variations such as oxygen tension, temperature, food availability and photoperiod can evoke biological changes at both the physiological and biochemical levels. It was observed that LDH specific activities were decreased in winter starved anuran amphibian tissues (Costa et al., 1983). During winter months, *O. nais* faces low temperatures and a lower food supply.

Fish in winter are not appreciably slower or more sluggish than those caught in summer. They compensate for temperature changes by appropriate alterations in metabolic rate. This is true not only for fish but for many other poikilothermic animals as well (Schmidt-Nielsen, 1993) such as the crabs *Hemigrapsus nudus* and *H. oregonensis* which can maintain their activity in winter (Rice and Armitage, 1974). However, maintaining the locomotory activity for an ectotherm which has the body temperature of its surroundings requires a several-fold increase in metabolic rate. This requires a high food intake (Schmidt-Nielsen, 1993).

In winter, however, the most alert of crayfish can find little food (Huxley, 1974a). Lack of feeding initiates the reduced metabolism of the starved decapod *Carcinus maenas* (Armitage and Wall, 1982). High LDH activities in this

study of nerve, heart and abdomen from each season implicate anaerobic glycolysis as an important source of energy in these tissues. Low temperature and lack of food may combine to cause a decrease in LDH specific activities from winter nerve, heart and abdominal muscle in this study since winter *O. nais* remain inactive (Rice and Armitage, 1974) and when disturbed they respond very sluggishly (personal observation). The reduced metabolic rate decreases the energy needs for these organisms which in turn enhances their ability to withstand the prolonged harsh winter environment.

The behavioral activity of an ectothermic animal varies with environmental temperatures (Costa et al., 1983). Thus, I hypothesized that changes in LDH specific activities of skeletal muscle related to temperature should be found. Such changes were found in spring, summer and winter. However, the LDH specific activity of fall acclimated animals which encounter a low temperature (5^0 C) is significantly higher (P=0) than those of spring and winter acclimated organisms. *O. nais* normally breeds in the fall (Rice and Armitage, 1974) during which time, the male seeks the female with great avidity in order to deposit the spermatozoa (Huxley, 1974b). The high LDH activity of abdominal muscle provides the energy required for the increased locomotory activity.

Gonad development in the reproductive cycle involves intensive metabolic activity. A number of teleost species

deplete their body fat reserves associated with the provision of energy for gonad growth (Wiegand and Peter, 1980). It was observed in *O. nais* that the hepatopancreas decreased in size as the gonads increased (Armitage and Topping, 1962). Observations in this study showed that the lipid reserves in the hepatopancreas of *O. nais* from fall acclimated animals appeared to be depleted (Personal observation). Many mammalian spermatozoa obtain some energy from glycolysis and have a predominantly aerobic metabolism (Mclndoe and Mitchell, 1978). The oxygen consumption of *O. nais* males increases during late summer and fall (Rice and Armitage, 1974). The increased oxygen consumption may be due to the utilization of lipid mobilized from hepatopancreas as an energy source for reproductive development and for maintenance of spermatogensis.

There was a relatively high LDH activity in the heart of fall acclimated animals in the present study. The heart rate of crustaceans increases when individuals become more active (Lockwood, 1968a). An increased locomotory activity in the breeding season may increase the heart rate to circulate more oxygen, substrates and metabolites. An increased anaerobic glycolysis which was reflected by the high LDH activity not only provides the heart sufficient energy for extra muscular activity but also reserves more oxygen for utilization by the reproductive organs.

Lactate production and LDH activity in the gills of crustaceans have been shown to be very low (Thabrew et al.,

1971; Dendinger and Schatzlein, 1973). The results of my experiments also showed that gill tissue from *O. nais* has relatively low LDH activity. The activities of mitochondrial TCA cycle enzymes were all high in crustacean *Pachygrapsus crassipes* gills. High activity of TCA cycle enzymes suggesting that gill tissue basically depends on aerobic respiration (Dendinger and Schatzlein, 1973). Burnett et al. (1980) also demonstrated that gill tissues from crustaceans take up a significant amount of oxygen compared to the total organismal O_2 uptake. These observations might explain the low LDH activity for pyruvate reduction in *O. nais* gills.

Although gill tissue is basically dependent on aerobic respiration, the LDH activities for pyruvate reduction were significantly higher in fall and winter than those in spring and summer. The high activity in fall suggests that oxygen may be selectively utilized by the gonad tissue requiring that gills increased anaerobic glycolysis to compensate for their own energy need during the breeding season.

The quantity of water which occupies the space left in the branchial chamber by the gills is very small. Since the respiratory surface offered by the gills is relatively large, the oxgen contained in this water must be rapidly exhausted, even when the crayfish is quiescent. Attached to the base of the second maxilla, there is a wide curved plate which fits against the projection of the head. This is termed the scaphognathite which can be readily moved

backwards and forwards. The repetition of the strokes of the scaphognathite increases the water flowing over the gill for gas exchange (Huxley, 1974b). There is a heart-scaphognathite coordination in crustaceans (McMahon and Wilkens, 1983). Heart rates in arthropods slow under low temperature conditions (DeFur and Mangum, 1979). Therefore, the decreased heart rate of winter acclimated *O. nais* should be accompanied by a decrease in the number of scaphognathite beats. In addition, there are six gills attached to the basal joints of the legs. When the animal exerts its muscles in walking, these gills are agitated and thus not only bring their own surface in contact with the water but produce the same effect upon the other gills (Huxley, 1974c). However, *O. nais* becomes inactive during winter. The decreased scaphognathite beats and the inactivity of this organism results in a reduced oxygen supply for gill tissue. Therefore, an increased LDH activity is used by gills to compensate for their energy needs during winter.

LDH activities of intestine and hepatopancreas before the treatment of protease inhibitors were very low; and those of the reverse reactions were undetectable. Protease was found to be produced in hepatopancreas and drained into midgut (van Weel, 1974). LDH might be hydrolyzed by proteases during homogenizing. After application of protease inhibitors, LDH activities for both pyruvate reduction and lactate oxidation from these two tissues

increased, especially in the intestine (Table 3). This indicates that LDHs of intestine and hepatopancreas were destroyed during extract preparation. However, after application of protease inhibitors, LDH activities from hepatopancreas were still very low. These observations suggest that LDH activities of hepatopancreas were originally low. This situtation was also found in the crab *P. crassipes* where glycolytic enzymes, including LDH of the hepatopancreas, were not detectable (Schatzlein et al., 1973). Popham and Dandy (1976) also found the crayfish *C. bartoni* possesses little anaerobic metabolism in the hepatopancreas. Phillips et al. (1977) found the respiratory quotient of hepatopancreas from crustaceans was 0.74 suggesting the energy source of this organ is mainly fat. β-oxidation of fatty acids takes place in the mitochondria where they are cleaved successively into acetyl CoA. Condensation with oxaloacetate produces citric acid which is oxidized via the Kreb's cycle. Fatty acid oxidation can occur only in the presence of carbohydrate which yields oxaloacetate. In the absence of oxaloacetate, ketobodies will be generated (Munday and Poat, 1971). Acetyl CoA is a powerful allosteric activator of pyruvate carboxylase (Voet and Voet, 1995). Accumulation of acetyl CoA will facilitate oxaloactate formation from pyruvate which in turn results in low LDH activity for lactate formation in *O. nais* hepatopancreas. Thus, fatty acids can

be oxidized since there no ketobodies have been found in crustacean hepatopancreas.

No incorporation of lactate into glucose and glycogen has been found in the hepatopancreas of this organ in crustaceans (Phillips et al., 1977). In crayfish, low LDH activity for lactate oxidation may indicate that there is no, or very little, gluconeogensis from lactate in the hepatopancreas, helping this organ to oxidize fatty acids more completely as gluconeogensis will reduce the level of oxaloacetate. So, low LDH activities for both pyruvate reduction and lactate oxidation helps the hepatopancreas to use fatty acids as an energy source.

Crustacean intestine has pronounced peristaltic and antiperistaltic waves for the movement of food in the gut (Lockwood, 1968b). Crayfish intestine has high LDH activity which may provide energy for this muscular process.

The mammalian nervous system is extremely dependent on the mitochondria-mediated oxidative metabolism and loses normal functional competence rapidly after oxygen deprivation (Atwood and Nguyen, 1995). Therefore, only LDH-1 and LDH-2 are present in the mouse brain (Markert and Ursprung, 1962). In the crustacean *C. opilio*, all five isozymes are present in nerve tissue (Angers et al., 1994) suggesting that anaerobic glycolysis is operative. In crustaceans, reliance of neural function on a well-maintained supply of oxygen has not been well documented (Atwood and Nguyen, 1995). High LDH activity in

O. nais nerve also indicates that an energy supply from anaerobic glycolysis for this tissue cannot be ignored. The conduction velocity of nerves is closely related to the temperature; the Q_{10} is about 1.6-2.5 (Wu et al., 1986). Nerve conduction slows at temperatures below 10^0 C (Schmidt-Nielsen, 1993). Comparison of nerve tissue shows higher LDH activities in summer acclimated crayfish. This higher LDH activity may provide more energy for the reestablishment of ion concentration gradients across the cell membrane which is needed for nerve conduction.

Like abdominal muscle and heart, nerve had the highest LDH activity in the fall when the temperature was 5^0 C. How the breeding season affects the activity and the metabolism of the nervous system in crustaceans has yet to be investigated so that the significance of the high LDH activity in this tissue during breeding season in *O. nais* needs further study.

Conclusion

Results from the electrophoresis show that LDH from *O. nais* possesses two isozymatic forms and these isozymes are tissue specific. No seasonality exists in the isozymatic patterns, but there is seasonality for LDH specific activity. It is surprising that LDHs in crayfish heart and nerve appear to be M-type. It would be interesting to determine the necessity for heart and nerve to depend on anaerobic glycolysis for energy requirement.

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