EVALUATING SURVIVAL OF THE CRAYFISH *Orconectes nais* EXPOSED TO HYPOXIC WINTER CONDITIONS

by

Charles Ephriam Kichler

B.S., Ohio State University, 1984

A MASTER'S THESIS

Submitted in partial fulfillment of the
requirements for the degree

MASTER OF SCIENCE

Division of Biology
Kansas State University
Manhattan, Kansas

1987

Approved by:
Harold E. Klaassen
Major Professor

TABLE OF CONTENTS

List of Figures

List of Tables

Acknowledgments

I would like to thank the Kansas Agricultural Experiment Station for funding and my committee members, Dr. Tim Seastedt, Dr. Fred Wilson and Dr. Harold Klaassen for their guidance and time toward this project. In addition, Dr. Harold Klaassen has patiently served as my advisor over the past 3 years.

Many people, my instructors, the office staff, and the store room staff have been indispensable. Without all of their support, this thesis would have been just another idea.

In addition, I have had a great deal of personal support from my parents, Ross and Caroline; my brother, David; and many of my friends both within and without the Division of Biology. Greg Farley was a great help with SAS. Dr. David Margolies was both a generous friend and a providing helpful suggestions througout this project. Finally, a note of very sincere thanks to Nicole Bramesco, who has read, critiqued, corrected this thesis, and provided loving support throughout this past year.

INTRODUCTION

This research examines the ability of the crayfish Orconectes nais to survive winter conditions in typical of Kansas farm ponds. The winter survival of this species is an important factor in developing crayfish aquaculture in the central states.

The culturing and harvesting of crayfish for human consumption is a rapidly expanding business in the southern United States (Anonymous, 1983) along with more traditional uses such as bait and scientific study. Huner (1985) estimated that there are 115,000 acres in aquacultural use in the southern states. Cultured and natural production produced 70-100 million pounds of Procambarus clarkii and P. astacus astacus in 1985. In 1982 estimated production was 55 million pounds, with a farm value of $27 million.

Currently, a mini-trend in Cajun/Creole cooking (Katz, 1987) has brought a new, potential aquacultural product to Kansas. The crayfish is being established as a gourmet food product. Rearing crayfish as a reliable crop is a largely unexplored problem in the central states, in spite of the existence of a large population of an appropriate-sized crayfish species, Orconectes nais (Huner and Avault, 1981). Research at Louisiana State University on crayfish aquaculture resulted in creating a highly profitable

industry from a previously nonexistent industry in just 20 years (Avault, 1986).

A similar investment into research at universities in the central states will be necessary to develop the crayfish industry in this region. One problem which needs to be researched is winter mortality of crayfish. This problem was suggested by Ingelin (1984) and by other research done by Dr. H.E. Klaassen (Unpublished data). Fish are known to experience winter mortality in ponds (Gablehouse et al., 1982). No studies have been done to demonstrate the overwintering conditions and capabilities of O. nais. Winter survival may be a keystone in culturing crayfish in the central states.

Conditions which create low dissolved oxygen (DO) in the winter are not predictable in extent or duration. Large variability exist from one winter to the next and from one pond to the next. To study winter conditions in a pond, an artificial environment that could be controlled to simulate conditions of low temperature and low oxygen concentration was necessary to circumvent the variation of nature. It allowed the removal of other possibly complicating biotic and abiotic factors along with making observation less difficult and potentially more accurate.

This thesis provides documentation of the development of an artificial environment that simulates winter conditions and two experiments that measure the ability of the crayfish, O. nais to survive simulated winter conditions.

Literature Review

This thesis examines the ability of the crayfish Orconectes nais to survive winter conditions in Kansas farm ponds. The common crayfish, O. nais, is a member of the class Crustacea within the phylum Arthropoda. This class has more than 1,100 freshwater species (Moffet, 1981a). The crayfish is in the order Decapoda, which has nearly 300 freshwater species in the United States, about 10% of which live in the Midwest (Moffet, 1981b). The genus Orconectes represents the largest group in Kansas (Williams and Leonard, 1952).

Crayfish are common aquatic organisms that are economically important. They are often used as objects of dissection in biology courses and for physiological experiments. More commonly, they are known for their outstanding quality as sportfish bait (Hauptman, 1984) since they are a natural prey of choice. They are gaining importance as a "seafood" product (Huner, 1978). Their influence has been spread by the recent "mini-trend of Cajun and Creole cuisine" (Katz, 1987). Crayfish are also considered an important component of the aquatic environment (Tack, 1942; Momot, 1966; Flint, 1974;

Bouchard, 1978; Gowing and Momot, 1979; Jones and Momot, 1981).

The culturing and harvesting of crayfish for bait and human consumption is a rapidly expanding business in the southern United States (Anonymous, 1983). Huner (1985) made the following estimates of production.

> There are about 115,000 acres of water in use for crayfish production. Most of this production occurs in Louisiana (105,000+ acres), but culturing is rapidly increasing in nearby states. The culturing in Louisiana along with wild areas produced 70-100 million pounds of Procambarus clarkii and P. astacus astacus. Other states with major production areas were Texas (8,000+ acres), Arkansas (500+ acres), Mississippi (500+ acres), and South Carolina (600+ acres). In 1980 production was 23.9 million pounds. Estimated production in 1982 was 55 million pounds, with a farm value of $27 million.

Wholesale bait prices for whole crayfish range from $1.50 to $15.00 per pound depending on the area of the country and seasonal demand (Huner and Avault, 1981).

Crayfish are very common in Kansas and can be abundant in some ponds (Jackson, 1965; Ingelin, 1984). Kansas could benefit from crayfish aquaculture, considering that the state ranks eighth in the nation in water acreage used for commercial fish production (Soil Cons. Service, 1979). Many of the commercial fish growers of the central states produce crayfish incidental to their fish production. Crayfish are sold as fish bait and in some cases are a substantial addition to their aquaculture income. An

example is Mr. W.E. Hartley of Kingman, Kansas, who sold approximately 1,200 pounds of crayfish for $3,500 in 1983. Crayfish culture in the central states has the potential for being developed into a substantial business for fish bait and human consumption since some species easily grow as large as southern species (Williams and Leonard, 1952; Huner and Barr, 1984).

A large literature base exists on the southern species Procambarus clarkii due to its high economic value. An excellent example of the literature is Huner and Barr's (1984) book on the culturing of P. clarkii. Unfortunately, P. clarkii literature is not applicable to more northerly species such as Orconectes nais. The southern species is an obligate burrower for reproduction, and most of its growth occurs during the cool seasons (fall, winter, and spring).

A potential crayfish industry has been developing in Kansas and the other central states without the advantage of any predetermined aquaculture techniques. The major obstacle has been a lack of basic information about crayfish such as their growth, optimum density, reproduction, and environmental requirements. This study was initiated with O. nais because of its abundance in ponds of the central United States (Williams and Leonard, 1952; Huner and Avault, 1981) and its size potential for

human consumption (Huner and Avault, 1981). One key environmental problem is the ability to survive winter in order to make culutre dependable and profitable.

There is a deceptively large amount of information on crayfish. For example they have been studied as experimental animal models (Massabuau et. al., 1984), as members of the aquatic ecosystem (Flint, 1974; Lorman and Mangnuson, 1978; Momot et al.,1978; Sommers and Steichy, 1986), and as an agricultural crop (Huner and Barr, 1980). The most commonly cited genera are the Astacus (Massabuau et al., 1984), Cambarus (Sommers and Steichy, 1986), Orconectes (Ingelin, 1984; Sommers and Steichy, 1986), Pasifasticus (Flint and Goldman, 1977), and Procambarus (Huner and Barr, 1980). However, only a small amount of the research has dealt with any of the facets of the problem of winter survival.

Investigations on the life history of a common central states crayfish, O. nais have been conducted by Williams and Leonard (1952), Jackson (1973), Pippet (1977), and Ingelin (1984). These studies dealt very little with winter conditions such as cold temperature and potentially depletion of dissolved oxygen (DO). However, Ingelin (1984) noted a drop in populations over the winter period. A similar trend of winter population decline has been noted during other studies at Kansas State University.

Several studies have been conducted on crayfish thermal tolerances, especially at abnormally warm conditions. Loring and Hall (1976), Cincotta (1979), Crawshaw (1983), and Taylor (1984) observed the temperature preferences of several species. Taylor (1984) worked with Procambarus clarkii, P. spiculifer, and Cambarus latimanus and found "all three species preferred the ambient temperature at which their peak activity occurred." Crawshaw (1983) found similar results with Orconectes immunis regardless of when they were fed. They selected 20-24°C water after a 20 min period. Acclimation temperatures ranged from 7 to 27°C. Cincotta (1979) found that the crayfish, O. obscurus preferred slightly warmer temperatures than their acclimation temperature, except at the 30 and 33°C levels where they moved to a cooler than acclimation zone. Loring and Hall (1976) used on O. causeyi a technique similar to Crawshaw's (1983) and found that they also went to a moderate temperature zone after 20 min.

Thorp (1978) looked at the relationship between temperature and antagonistic behavior in Cambarus latimanus. He tested 9.5, 14, 22, and 30°C in the summer and 9.5 and 22°C in the winter. In both seasons, the colder tested temperature caused more antagonistic behavior. This study showed that several species,

including several members of the genus Orconectes are able to withstand and acclimate to a wide range of warm temperatures. Claussen (1980) found with Procambarus clarkii and Orconectes rusticus that "the thermal tolerance limits of crayfish seem to be quite resistant to factors other than acclimation temperature."

A large portion of the literature dealing with problem of extreme heat coupled with low DO is from the southern states. In the South, low DO is due to the increased biological activity, primarily bacterial, within the warmed water. Similar problems occur at any latitude in shallow bodies of water. Oxygen deprivation reaches its extreme in the morning. At this point, an entire night of respiration has taken place and photosynthetic oxygen production has not yet begun. Even though summer morning fish kills are a problem in Kansas (Gabelhouse et al.,1982), crayfish are not as susceptible partially due to their ability to come out of the water. Unfortunately, most literature is concerned only with Procambarus clarkii, the principal species of southern crayfish aquaculture.

The thermal tolerance of crayfish has also been examined at low temperatures. Thorp (1978) found that antagonistic crayfish behavior was inversely proportional to temperature between 9.5 and 30°C. His results indicate that crayfish can maintain themselves at lower

temperatures. Aiken (1969) looked at the relationship between ovarian maturation and temperature. He found that 4°C water was necessary for ovarian maturation and a successful hatch in the crayfish Orconectes virilis. Kivivvori (1983) looked at the activity of walking at various acclimation temperatures. He found that, within the range of naturally occuring temperatures, the crayfish Astacus astacus could develop spontaneous walking activity once it had been acclimated beyond 11 days at the test temperature.

Crayfish have been shown to have limits of thermal tolerance. Cox and Beauchamp (1982) using Cambarus bartoni (Fabricus) observed that juveniles were susceptible to even brief pulses of 40°C water. This study was undertaken to look at the critical impact of power plant cooling water. White (1983) explored the LD50 of Procambarus darhi using a various combinations of high temperature water and exposure time. Acclimation temperature was found to be a significant variable. The LD50 occurred at 34-40°C. At all temperatures predicted LD50 times were 2 to 3 times longer when the crayfish had been acclimated at 25°C instead of 10°C. Intolerance to high temperatures has been a major problem in the southern states (Huner and Barr, 1980) and with power plant effluents (Cox and Beauchamp, 1980; White, 1983). Aiken (1967) examined the lower end of

thermal tolerance. He could not revive frozen Orconectes virilis, yet he noted that the crayfish commonly found in natural waters near 0°C.

Several studies have documented the oxygen consumption of crayfish. Most notably, Wiens and Armitage (1961) used 16, 24, 30 and 35°C water at full, one-half, one-quarter, and one-eighth oxygen saturation. A flow-through chamber was used to house the experimental animals. The Orconectes immunis and O. nais used in this study were all taken from Douglas County, KS. They found high correlations between the dependent consumption rate and the independent variables of saturation level, temperature, and individual body weights. They found that O. nais was less tolerant to 35°C and 25% saturated DO then O. immunis. The difference between the two species accounts for the former's colonization of ponds and the latter's tendency to inhabit more temporary bodies of water such as roadside ditches. Unfortunately, this study yields little information on winter survival because only summer temperatures were tested.

Armitage and Wall (1982) found respiratory rate of Orconectes nais to vary greatly depending on whether they were starved or un-starved. For example, a fed 35 g crayfish would have a consumption rate of 0.133 ml/g/h versus starved crayfish would have a consumption rate of 0.041

ml/g/h, both at 30°C. A similar pattern of greatly increased oxygen consumption with feeding has also been noted for other crustaceans, such as the tiger prawn, Penaeus esculentus (Dall and Smith, 1986). Increased metabolism can also be caused by the high energy demands of molting and associated tissue growth (Rice and Armitage, 1974).

Morrissy et al. (1984) found the oxygen consumption of the crayfishes, Maron tenuimanus and M. albidus in closed system test to be 0.074 ml/g/h (all conversion from weight to volume are based on idea gas law). Spencer (1984) found the oxygen consumption using the equation:

$$M = KW^2$$

where M = total oxygen consumed per time unit
W = body weight
K,b are constants

With Orconectes propinquis, he found daily consumption to vary from 0.294 to 0.420 ml/g/h and from 0.378 to 0.882 ml/g/h at 24°C over the duration of the experiment. His results showed a wide range of variation between days and were one order of magnitude greater than those from previous authors' experiments. No explanation was given for the differences because Spencer was looking for the difference between treatments with and without Aquashade, a dye-type of aquatic herbicide, on the same days.

McMahon et al. (1974) tested the long-term respiratory response of Orconectes virilis to hypoxic stress. Their experiment was done to observe the physiological responses. They cite an initial increase in scaphognathite beat (the rate that small appendages used to circulate water across the gills move) and volume per beat during the initial 72 h followed by lowering to base levels for the duration of the experiment (240 h). The increase occurred during the initial administration of both normoxic water (water within an expected range of saturation at the given temperature) and hypoxic water. The partial pressure of oxygen used was 30 Torr (~2.4 mg/L DO at 20°C).

No studies have been done to demonstrate the over-wintering conditions and capabilities of Orconectes nais. This species of crayfish is already ecologically important in Kansas and could become economically important with future developments in aquaculture research. Winter survival is a potentially important factor in the feasibility of crayfish aquaculture in the central states. Winter represents a period of high mortality for crayfish (Ingelin, 1984). Until this problem of winter survival is understood, crayfish winter mortality will represent lost aquacultural potential production. This thesis is a first step in solving the problem of winter survival.

The Survival of the Crayfish Orconectes nais Under Simulated Winter Conditions

Introduction

Crayfish have become a major agricultural industry in the southern states over the last 20 years through an aggressive research program (Avault, 1986). The crayfish, Orconectes nais, has potential as an agricultural crop in the central states based on its size (Huner and Avault, 1981) and abundance (Jackson, 1965; Ingelin, 1984). Problems unique from the South face the central states in developing crayfish aquaculture. The central states have a very different climate and growing season. A severe winter mortality may be a barrier to profitable culture in this region. The mortality may be caused by a lack of dissolved oxygen (DO).

Two experiments were run to document the ability of O. nais to survive at common winter levels of DO and temperature. Previous investigations examined only short-term O_2 consumption rates (Weins and Armitage, 1961; Armitage and Wall, 1982; Morrissy et al., 1984; Spencer, 1984), temperature preferences (Loring and Hall, 1976; Cincotta, 1979; Crawshaw, 1983; Taylor, 1984), or temperature tolerances (Aiken, 1967; Aiken, 1969; Claussen, 1980; Cox and Beauchamp, 1982; Kivivvori, 1983; White,

1983). An investigation into the long-term respiratory response of O. virilis to hypoxia at 20°C (McMahon et al., 1974) explored a similar area, but at a summer temperature.

The two experiments were done to measure the survival of crayfish during a harsh winter. The harshest winter conditions occur when a layer of ice is covered with snow preventing O_2 diffusion and photosynthetic light from entering the water. The crayfish survival was measured in a winter simulation system. The simulation allowed the survival of crayfish to be measured at low DO and temperature. The initial experiment measured survival at two cold temperatures and two low DO levels. The second experiment measured survival at 4°C and near 0 mg/L DO. From each experiment, two valid models of crayfish survival were created. These models are then related to practical implications of overwintering crayfish in the central states.

METHODS AND MATERIALS

Experimental Preparation

Young of the year (YOY) crayfish O. nais were seined in the fall of 1985 and fall of 1986 from a pond located on Kansas State University Pastures. The first experiment used crayfish from both years and the second only used crayfish from 1986. The pond had been noted for its

excellent production of crayfish, even with some winter kill problem (Unpublished data). It is a typical Kansas farm pond with turbid water (< 0.5 m Sechi disk depth) due to livestock. The captured crayfish were kept in a fiberglas tank.

All tanks used were either a Frigid Units, Inc. Min-O-Cool tank (model MT-700) or a Living Stream tank (model LS-700). Both tanks had a working volume of 500 L. The former was used to hold crayfish at ambient temperatures and the latter was used to hold crayfish at below-ambient temperatures. The Living Stream tank was preferred for its better cooling with its false bottom in conjunction with a Water Chiller Unit (model D1-33).

All experiments were furnished with dechlorinated city water. Water at approximately 18°C in the summer and 16°C in the fall continuously flushed the tanks. Stock crayfish were fed 30% protein sinking catfish diet. The room was maintained on an 8h/16h light/dark day to simulate a winter photoperiod with incandescent lights on a timer. Previous experience had proven this to be a successful method of holding crayfish, although some loss was experienced due to cannibalism upon the freshly molted crayfish.

A simulation system was constructed for both experiments. Four experimental 20 L chambers were constructed from Plexiglas. Each chamber was constructed as

detailed in Figure 1 with 6.35 mm (1/4 inch) Plexiglas. A removable Plexiglas portal cover was secured with wing nuts and bolts. The portal was sealed with foam weather stripping coated with silicone sealant and a piece of plastic wrap. Water was circulated from one end to other through tubing with a Silent Giant Garden/Fountain pump (model 42-A). The assembled chamber is shown in Figure 2.

Ports were provided to remove water samples, collect temperature data, and administer an artificial atmosphere. Chamber water samples were taken at the inlet end of the chamber from a tee-fitting using the positive pressure. The temperature was measured to the nearest 1/10°C with the thermometer mounted in the top of the chamber.

The chambers were then almost completely submerged in a tank cooled by a water chiller. The surrounding bath cooled the chamber to a selected temperature determined by the water chiller thermostat. Figure 3 shows the arrangement from a side view.

A predetermined concentration of O_2 in N_2 was administered through aquarium tubing to each chamber. One 60 mm Cole-Parmer flowmeter (model FM012-10ST) per chamber was used to maintain a constant flow of gas at 95 ml/min. The gas took a countercurrent path of the water for maximum efficiency (Vander et al.,1980). Each flowmeter was fed off a two-stage oxygen regulator adapted to the proper fitting.

Gas exited the chamber through a one-way gas trap mounted on top of the chamber.

The gas controlled the DO in the water within each chamber by creating an artificial atmosphere with a selected partial pressure. Henry's Law states that the partial pressure of a gas in the atmosphere is proportional to its concentration in a solution at a given temperature (Moore, 1980). Custom-blended gases replaced the "normal" atmospheric air and thereby changed the saturation concentration of oxygen in a chamber. A mixture of 0.9% O_2 in N_2 or pure N_2 was administered to create the two levels of DO used in each experiment.

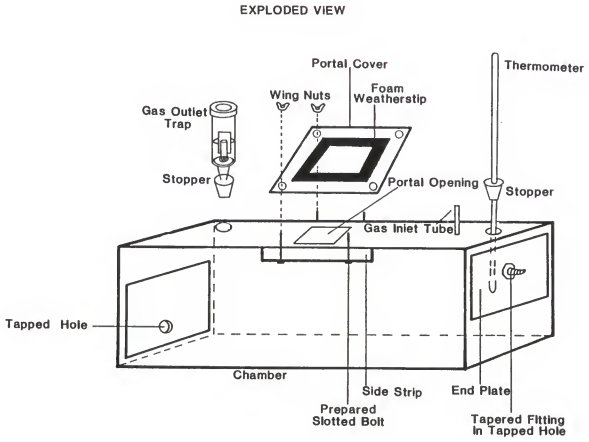

Figure 1. An exploded view of a chamber with all fittings.

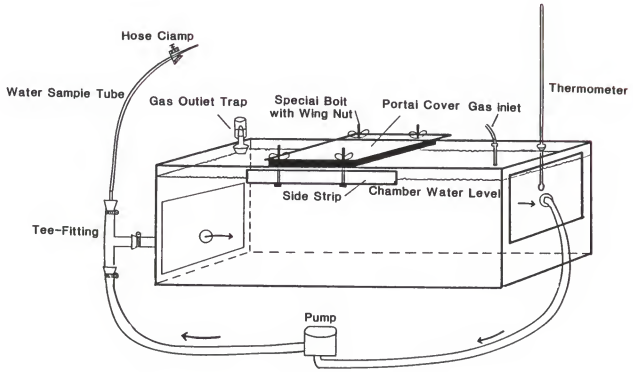

Figure 2. The chamber arranged in run configuration with circulatory and gas lines installed.

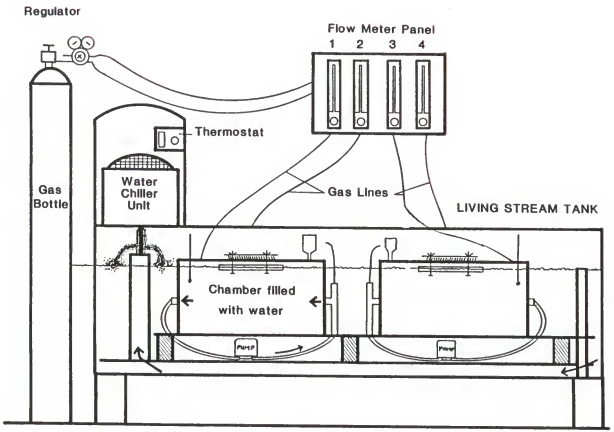

Figure 3. A side view of the chambers in the cooling tank with the water chiller unit and the gas administration equiment installed.

Experiment one

Two acclimation tanks were used. The first acclimation tank had a target temperature of 10.0°C. Plastic screens topped with 10 cm galvanized tin strips at each end of the tank prevented the crayfish from being swept into the water chiller unit. The second and final acclimation tank had a target temperature of 4.0°C. The crayfish in this tank were held in banks of 10 x 10 cm individual 6.7 mm (1/4 inch) mesh hail screen cages. The crayfish in this tank were NOT fed.

Crayfish were always acclimated in each acclimation tank for a minimum of 14 d. Kivivvori (1983) had found acclimation of at least 11 d to be sufficient in an experiment examining nerve system responses.

Each experimental run began with the chamber being filled with dechlorinated city water and sealed without crayfish being present. N_2 was administered to lower the water's DO from its normal range of 8 - 10 mg/L to less than 1 mg/L. This process took approximately 4 d during which the DO was monitored daily. The water chillers ran throughout this period to lower the chamber to a selected temperature. Once the chamber had dropped below 1 mg/L, either 0.9% O_2 in N_2 gas was used to drop the chamber water to a targeted 0.5 mg/L or N_2 was continued until the DO was

less than 0.04 mg/L (the lowest discernible value). The second stage administration took an additional 2 d.

Once targeted temperatures and DO concentrations had been reached, 10 healthy, acclimated crayfish (O. nais) were added to each chamber. All crayfish passed through the two acclimation tanks. A two-stage acclimation was used to lessen the system shock of the crayfish when being transferred between different temperatures.

Experimental runs were made at either 0.0 or 0.5 mg/L DO and 2.0 or 4.0 C with 4 replicates per run. Each chamber was considered a replicate. Run 1 was an exception with only 2 chambers in use. A total of 6 runs of approximately 11½ d were made between May 13, 1986 and February 9, 1987. Temperature data were collected daily and the DO concentrations were analyzed from 300 ml samples drawn on alternate days. Previous to water sampling, each biological oxygen demand (BOD) bottle was filled with N_2 to minimize oxygenation of the sample. A modified Winkler Method (APHA, 1980) was used to determine the DO. The lowest discernible value of DO with this method was between 0.04 mg/L and 0.02 mg/L. The number of dead and live crayfish was counted at the 275 h.

Experiment two

A single-stage acclimation of >14 d was used employing the previously mentioned final stage acclimation tank at 4.0°C without food. The same chambers as in experiment one were used. The preparation method for the chambers was modified to lessen turnover time as follows. After the chambers had been submerged in the cooling tank (Figure 3), each chamber was flushed for 1 d with water from the surrounding tank. The tube from the chamber water exhaust was slid off and placed into the surrounding water bath forcing water through the chamber and out of the top (note the direction of flow in Figure 2). On the following day, the exhaust was reconnected such that the water came to the top and the portal cover was closed. N_2 was administered causing a stream of N_2 bubbles in the chamber water. After 2 d water was released through the water sampling tube to lower the level approximately 1 cm below the top. The N_2 continued to flow through the chamber's atmosphere for another day to assure that the water was not supersaturated with N_2 . The water chillers ran throughout this period to lower the chamber temperature to the selected temperature.

At the end of initialization, the chambers were at ≤0.04 mg/L DO and approximately 4.0°C. Ten healthy, acclimated, individual crayfish, O. nais, were added to each chamber. Chambers were then sealed for 3, 6, 9 or 12

days. Temperature and DO was collected using the method from experiment one. The number of dead and live crayfish were counted at the conclusion each chamber run. Crayfish were considered alive if they moved their appendages within a half hour after being removed from the chamber at the conclusion of a run.

Data analysis

Lotus 1-2-3 version 1.2 was used to calculate the values in Table 1. SAS versions 82.3 and 5.93 at the Kansas State University Computing Center were employed to analyze do all further statistical calculations including ANOVA and linear models in both experiments one and two.

Results and Discussion

Both experiments were dependent on the biologically based assumption that DO was the critical factor for survival in very cold water. Death due to waste products was not eliminated, except by the assumptions that 1) at low temperatures, metabolisms in poikilotherms metabolic processes are slowed as is waste production; 2) starved crayfish would produce insignificant amounts of waste; 3) a chamber of 20 L would sufficiently dilute any waste produced; 4) the gas passing through the system would remove waste gases (such as CO_2); 5) the water in Kansas is well buffered.

Experiment one

A total of 5200 chamber hours were logged during this experiment of which 4442 chamber hours yielded valid survival data. The data were considered invalid only if a chamber's DO concentration displayed extremely wide variation. The extreme variation corresponded directly to known physical problems from blown gas delivery lines, gas leaking through the chamber lids, or poor circulation due to a crayfish getting stuck in the chamber outflow. The runs with valid survival data are grouped by targeted DO and temperature in Table 1. Figure 4 shows a three dimensional plot of average survival at the two DO concentrations and two temperatures.

Figure 5 shows the survival versus DO concentration. Figure 6 shows survival versus the Ln (Log_e) of DO. Both figures include the actual data, a regression equation, a regression line, and the 95% confidence interval.

Table 1. Survival by run, with mean, SD and n of DO and temperature of each run. All runs were for 275 h.

Target DO = 0.0 mg/L
Target temperature = 2.0°C

Run. Chamber	Survival	DO ± SD	n	Temp.± SD	n
2.2	0.00	0.02±0.00	6	2.03±0.11	11
2.3	0.00	0.02±0.00	6	2.10±0.12	11
2.4	0.00	0.02±0.00	6	2.23±0.13	11
3.3	0.11*	0.06±0.07	6	2.15±0.09	11
3.4	0.11*	0.02±0.00	6	2.15±0.09	11
Average	0.044±0.006				

* - only 9 crayfish started in these runs.

Target DO = 0.5 mg/L
Target temperature = 2.0°C

Run. Chamber	Survival	DO ± SD	n	Temp.± SD	n
4.1	0.50	0.28±0.13	6	1.72±0.39	11
4.2	0.70	0.26±0.13	6	1.95±0.14	11
4.3	0.70	0.39±0.11	6	2.30±0.06	11
Average	0.633±0.115				

Target DO = 0.0 mg/L
Target temperature = 4.0°C

Run. Chamber	Survival	DO ± SD	n	Temp.± SD	n
5.1	0.00	0.03±0.01	6	3.18±1.44	11
5.2	0.20	0.03±0.01	6	3.13±1.46	11
5.3	0.00	0.03±0.01	6	3.28±1.42	11
Average	0.067±0.115				

Target DO = 0.5 mg/L
Target temperature = 4.0°C

Run. Chamber	Survival	DO ± SD	n	Temp.± SD	n
6.1	0.90	0.22±0.07	6	3.76±0.88	11
6.3	0.90	0.24±0.06	6	3.75±0.84	11
6.4	1.00	0.32±0.04	6	3.73±0.85	11
Average	0.933±0.058				

Experimental run dates.

1. May 13, 1986
2. May 23, 1986
3. Jun. 29, 1986
4. Sep. 10, 1986
5. Nov. 26, 1986
6. Jan. 30, 1987

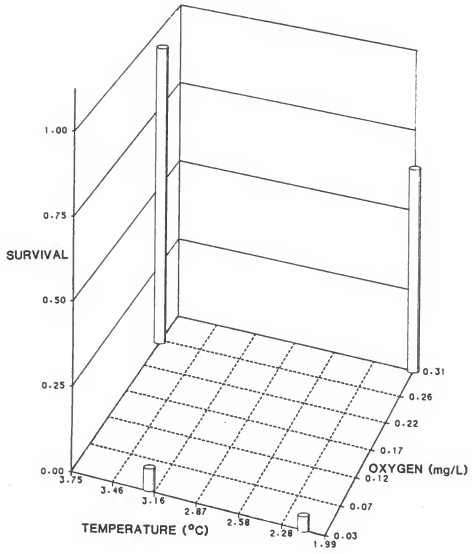

Figure 4. A 3-dimensional plot of mean survival at mean DO concentration by mean temperature.

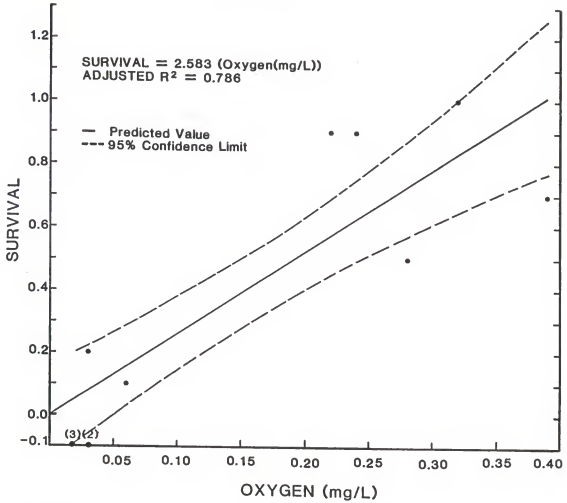

Figure 5. A graph of survival versus DO concentration with a predicted value line and a 95% confidence interval.

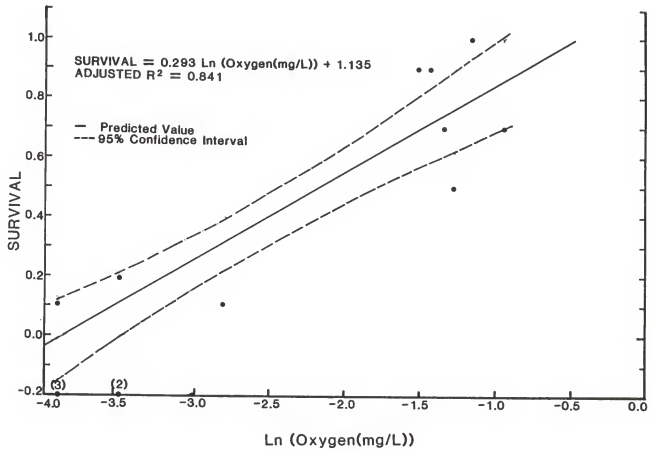

Figure 6. A graph of survival versus Ln DO concentration with a predicted value line and a 95% confidence interval.

Temperature had a much wider variation than expected. The refrigeration units were unable to maintain a narrow temperature tolerance due to the extremely low temperature demanded and a summer voltage drop. The mean temperature of each chamber did not directly affect survival ($r=0.445$). An interaction between temperature and DO may have occurred as indicated in Figure 4.

Oxygen concentration was a significant factor in survival. Two clusters of data points were produced (Figure 4 or 5). This pattern would only allow a linear modeling technique to be used and could not rule out other more complex models. The mean DO was highly correlated with survival ($R^2=0.786$). A Ln of the mean DO had an even higher correlation with survival ($R^2=0.841$). Figures 4 and 5 show the actual data, the predicted mean line, and the 95% confidence interval.

Other linear models using both temperature and DO were not viable for two major reasons. First, the mean temperatures of the chambers were not significantly different due to the wide individual variation. Secondly, survival was positively correlated with temperature. If lower temperatures create reduced metabolic O_2 demand in a poikilothermic crayfish, then the correlation should be inverse to temperature. Therefore, based on these data

alone, no useful model was found which incorporated temperature with DO concentration.

Experiment two

Three runs were made for each time period of 3,6,9 and 12 d. All 12 runs were made between April 9 and May 9, 1987. Maximum and minimum temperatures were 6.8°C and 3.0°C, respectively. Maximum and minimum DO were 0.10 mg/L and 0.04 mg/L, respectively. Table 2 contains the length of the run, the mean temperature, the mean oxygen concentration, and the survival at the end of the time period. The survival versus time of exposure is plotted arithmetically and logarithmically in Figures 7 and 8.

The temperature data varied widely over time (Table 2). This variation is a direct result of the extreme stress placed on the Frigid Units, Inc. water chiller, which was not designed for low variation in temperature. The only variation in DO was during three of the runs. This was due to a shipping error where an empty bottle of N_2 was accidentally supplied. During these three runs, the DO measurement was taken just before N_2 was restarted. A measurement at this point, represents the maximum DO concentration. All previous measurements had been taken at unbiased intervals. The bias of selecting the sample at the known high point to be included in the mean, creates the worst mean (highest possible). If the sampling times

had been totally at random or totally regular, the mean would not be biased and therefore more representative.

Exposure to the conditions of winter hypoxia, extremely low oxygen concentration and low temperature, was inversely correlated with survival. Linear models were generated using both time in d and Ln(d). The logarithmic model produced a higher correlation (R^2=0.719 vs. R^2=0.626). The logarithmic transformation is the better model if the ability to survive is a polygenic trait and normally distributed in the population. An extension of the predicted value line at t=0 would yield approximately 100% survival.

Two factors other than exposure time could have potentially influenced survival. Temperature had no correlation (r = 0.423) with survival. The length of the starvation on the crayfish beyond the initial 14 day period had no correlation (r = -0.079) with survival.

Table 2. The survival for each run at time (d), temperature mean and SD, DO concentration mean and SD.

Run	Time(d)	Temp.(°C) Avg.± SD	n	DO (mg/L) Avg.± SD	n	Survival
1	3	5.43±1.48	4	0.040±0.000	3	0.8
2	3	3.95±0.47	4	0.040±0.000	3	0.6
3	3	4.85±0.34	4	0.040±0.000	3	0.4
4	6	5.09±1.09	7	0.040±0.000	4	0.3
5	6	4.03±0.60	7	0.040±0.000	4	0.3
6	6	4.17±0.56	7	0.040±0.000	4	0.0
7	9	4.96±0.70	10	0.040±0.000	6	0.1
8	9	4.11±0.36	10	0.050±0.017	6	0.1
9	9	4.09±0.52	10	0.051±0.023	6	0.2
10	12	4.66±0.84	13	0.040±0.000	7	0.1
11	12	4.36±0.72	13	0.040±0.000	7	0.0
12	12	4.06±0.55	13	0.045±0.009	7	0.0

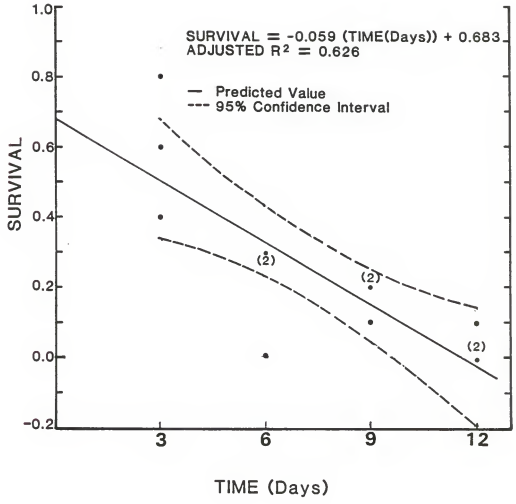

Figure 7. A plot of the relationship between survival and time of exposure in days. Actual data, mean, and 95% confidence intervals are given.

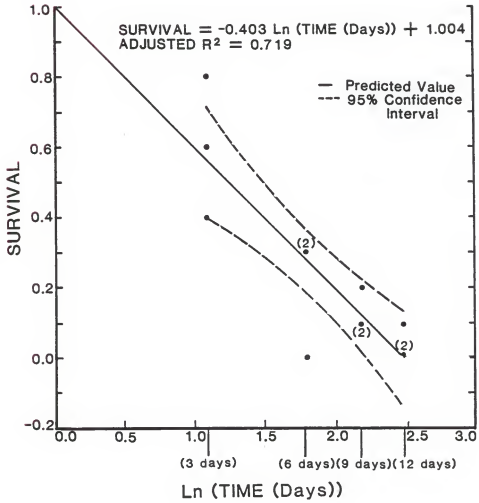

Figure 8. A plot of the relationship between survival and time of exposure in ln(days). Actual data, mean, and 95% confidence intervals are given.

Conclusion

This research was designed to explore the problem of winter survival of the crayfish Orconectes nais under anoxic winter conditions. A simulation system was developed to circumvent many of the distracting variations possible in nature. This system has the potential for being a more realistic substitute for the aquarium in bioassays through the systems control of DO and temperature.

Experiments one and two demonstrate the effect of low DO on the firstyear age class of O. nais during the winter. Experiment one varies the level of DO at two different temperatures. The temperature control was not sufficient to allow a separation between the two temperatures targeted. Survival could be predicted by the DO concentration. An arithmetic and a logarithmic scaled linear model predicted a 100% survival over 12 d at 0.387 mg/L and 0.632 mg/L, respectively. Survival required some DO, but these levels were very low. This was due to the low temperature (Armitage and Wall, 1982; Weins and Armitage, 1961) and starvation (Armitage and Wall, 1982; Dall and Smith, 1986).

Experiment two attempted to remove some of the importance of temperature by: 1) running at a higher temperature (4°C vs. 2°C); 2) making time the independent

variable. The experiment was conducted with extremely low DO concentrations (0.04 mg/L). The DO remained constant, excluding an incident with a unfilled "full" gas bottle. Survival was found to be inversely proportional to time using both arithmetic and logarithmic time scales. The logarithmic model was the better model because its better fit (adjusted R^2=0.719) and its passage through 100% survival at time=0. Any exposure to an extremely low level of DO would cause a lower survival.

A technique for simulating the environment beneath snow covered ice was used in this research. It can be used to begin to form some guidelines for aquaculture. Field validation would be a next step in further solidifying the necessary techniques for overwintering crayfish.

Based the results of these experiment, no loss of survival would be incurred at 0.65 mg/L DO extrapolating the Ln scaled linear model at 12 d. Because of the over-extension of this model, a practical recommendation with a margin of safety would suggest maintaining a DO at 1 mg/L or greater in the winter. Crayfish also showed an ability to withstand very low DO for brief periods of time with minimal mortality. These results suggest that winterkill due to low DO can be avoided. Because only extremely low DO appears to be a problem, aquaculturist should have adequate time to take corrective actions. In addition,

maintaining a DO above 0 mg/L is sufficient to prevent the breakdown of the oxidized microzone and its associated problems (Boyd, 1979; Wetzel, 1983).

Many techniques are available to increase the DO (Stickney, 1979). In general, a total ice cover of a production pond is not an acceptable practice. A total cover of ice and snow is usually necessary to drop the DO to 0 mg/L. Simply removing the snow may be sufficient to prevent winterkill (Gablehouse et al., 1982). Feeding crayfish just prior to freezing conditions should be discouraged. The unconsumed food could increase the biological and chemical oxygen demand. In addition, starved crayfish show a lower oxygen consumption at a given temperature (Armitage and Wall, 1982) and lowers their minimum tolerance to low DO concentrations. This research begins to provide the necessary background for culturing crayfish in the central states.

Literature Cited

Aiken, D.E. 1967. Orconectes virilis: Survival in a region with severe winter conditions. Canadian Journal of Zoology. 46:207-217.

Aiken, D.E. 1969. Ovarian maturation and egg laying in the crayfish Orconectes virilis: influence of temperature and photoperiod. Canadian Journal of Zoology. 47:931-935.

APHA (American Public Health Association), American Water Works Association, and Water Pollution Works Association. 1980. Standard methods for the examination of water and waste water, 13th ed. New York: American Public Health Association, Inc.

Anonymous. 1983. Aquaculture Magazine 1984 buyers guide. Asheville, NC. p. 6.

Armitage, K.B., and T.J. Wall. 1982. The effects of body size, starvation and temperature acclimation on oxygen consumption Orconectes nais. Comp. Biochem. Physiol A. Comp Physiol. 73(1):63-68.

Avault, Jr., J.W. 1986. Aquaculture potential in the United States. Aquaculture Magazine. 12(5):43-45.

Bouchard, R.W. 1978. Taxonomy, distribution, and general ecology of the genera of North American crayfishes. Fisheries. 3(6):11-16.

Boyd, C.E. 1979. Water quality in warmwater fish ponds. Auburn University Agricultural Experiment Station. Auburn, AL. 359 p.

Cincotta, D.A. 1979. Use of ahorizontal trough in temperature preference test for crayfish. Titles and Abstracts from the 27th Annual meeting of the North American Benthological Society, April, 1979. Erie, PA.

Claussen, D.L. 1980. Factors affeacting the temperature tolerance of crayfish. American Zoology. 20(4):734.

Cox, D.K., and J.J. Beauchamp. 1982. Thermal resetance of juvenile crayfish, Cambarus bartoni(Fabricus): Experiment and model. American Midland Naturalist. 108(1):187-193

Crawshaw, L.I. 1983. Effects of thermal acclimation and starvation on temperature selection and activity in the crayfish Orconectes immunis. Comp Biochem Physiol A Comp Physiol. 74(2):475-477.

Dall, W., and D.M. Smith. 1986. Oxygen consumption and ammonia excretion in fed and starved tiger prawns, Panaccus esulentus Haswell. Aquaculture. 55:23-33.

Flint, R.W. 1974. Seasonal activity, migration, alnd distribution of the crayfish, Pasifasticus leniusculus in Lake Tahoe. American Midland Naturalist. 97:280-292.

Flint, R.W., and C.R. Goldman. 1977. Crayfish growth in Lake Tahoe: effects of habitat variation. Journal of Fish. Res. Board of Canada. 34:155-159.

Flint, R.W. 1974. Seasonal activity, migration and distribution of the crayfish, Pasifasticus leniusculus, in Lake Tahoe. American Midland Naturalist. 97:280-292.

Gabelhouse, D.W., R.L. Hager, and H.E. Klaassen. 1982. Producing fish and wildlife from Kansas ponds. Kansas Fish and Game Commission., Pratt, KS.

Gowing H., and W.T. Momot. 1979. Impact of brook trout (Salvelinus fontinalis) predation on the crayfish Orconectes virilis in 3 Michigan lakes. Journal of the Fisheries Research Board of Canada. 36(10):1191-1196.

Hauptman, C. 1984. The irresistable crayfish. Field and Stream. 89(8):68,104-106.

Huner, J.V. 1978. Exploitation of freshwater crayfish of North America. Fisheries. 3(6):2-5.

Huner, J.V. 1985. An update on crawfish aquaculture. Aquaculture Magazine. 11(4):33-40.

Huner, J.V., and J.W. Avault, Jr. 1981. Producing crawfish for fish bait. Sea Grant Publication No. LSU-TI-001. Louisiana State University, Baton Rogue, LA.

Huner, J.V., and J.E. Barr. 1980. Red swamp crawfish: biology and exploitaion. Sea Grant Publication no. LSU-T-80-001 LSU Center for Wetland Resources. Baton Rogue, LA.

Huner, J.V., and J.E. Barr. 1984. Red swamp crawfish: biology and exploitaion. Sea Grant Publication no. LSU-T-80-001 LSU Center for Wetland Resources. Baton Rogue, LA.

Ingelin, M.E. 1984. Growth and distribution of crayfish in Kansas farm ponds. Master's Thesis. Kansas State University, Manhattan, KS.

Jackson, H.P. 1965. Observations on the crayfish Orconectes nais (Faxon) in two Kansas ponds. Master's Thesis. University of Kansas, Lawernce, KS.

Jones, Jr, P.D., and W.T. Momot. 1981. Crayfish productivity, allocthchy, and basin morphometry. Canadian Journal of Fisheries and Aquatic Sciences. 38(2):175-1983.

Katz, F.R. 1987. In Test Marketing. Cereal Foods World. 32(2): 206.

Kivivvori, L. 1983. Temperature acclimation of walking in the crayfish Astacus astacus. Comp. Biochem. Physiol. A Comp. Physiol. 75(3):375-378.

Loring, M.W., and L.G. Hall. 1976. Temperature selection and shelter utillization of the crayfish Orconectes causeyi. Southwestern Naturalist. 21(2):219-226.

Lorman, J.G., and J.J. Mangnuson. 1978. The role of crayfishes in aquatic ecosystems. Fisheries. 3(6): 8-10.

Massabuau, J.C., P. DeJours, and Y. Sakakibara. 1984. Ventilatory CO2 drive in crayfish: influence of oxygen consumption leval and water oxygenation. Journal of Comparative Physiology, B. 154:65-72.

Minshall, G.W., K.W. Cummins, R.C. Petersen, C.E. Cushing, D.A. Bruns, J.R. Sedell, and R.L. Vannote. 1985. Developments in stream ecosystem theory. Canadian Journal of Fisheries and Aquatic Sciences. 42: 1045-1055.

Moffet, M.F. 1981a. Introduction to the crustacea. Pages 38-41 in D.G. Huggins, P.M. Liechti, L.C. Ferrington, editors. Guide to the freshwater invertebrates of the Midwest. State Biological Survey of Kansas, University of Kansas, ed. Technical Publication No. 11 of the State Biological Survey of Kansas and The University of Kansas.

Moffet, M.F. 1981b. Decapoda (crayfish and shrimps). Pages 44-46 in D.G. Huggins, P.M. Liechti, L.C. Ferrington, editors. Guide to the freshwater invertebrates of the Midwest. State Biological Survey of Kansas, University of Kansas, ed. Technical Publication No. 11 of the State Biological Survey of Kansas and The University of Kansas.

Momot, W.T. 1966. Population dynamics and productivity of the crayfish Orconectes virilis in Marl Lake. American Midland Naturalist. 78(1):55-81.

Momot, W.T., Gowing, H., and Jones, P.D. 1978. The dynamics of crayfish and their role in ecosystems. American Midland Naturalist. 99:10-35.

Moore, W.J. 1983. Basic physical chemistry. Prentice-Hall, Inc. Englewood, NJ.

Morrissy, N.M., N. Caputi, and R.R. House. 1984. Tolerance of marron (Cherax tenuimanus) to hypoxia in relation to aquaculture. Aquaculture. 41:61-74.

Pippet, M.R. 1977. Mating behavior of the crayfish Orconectes nais (Faxon 1885)(Decapod, Astracoidea). Crustaceana. 32(3):265-271.

Rice, P., and K. Armitage. 1974. Effect of photoperiod on oxygen consumption of the crayfish Orconectes nais. Comp. Biochem. Physiol. 47A:261-270.

Soil Conservation Service. 1979. Aquaculture survey, USDA Report.

Sommers, K.M., and D.P.M. Steichy. 1986. Variable trappability of crayfish associated with bait type, water temperature and lunar phase. American Midland Naturalist. 116(1):36-44.

Spencer, D.F. 1984. Oxygen consumption by the crayfish Orconectes propiquis (Girard) exposed to Aquashade. Bulletin of Evironmental Contaimination Toxicology. 33:373 -378.

Stickney, R.R. 1979. Principles of Warmwater agriculture. John Wiley and Sons. New York, NY.

Tack, P.I. 1942. Life histories and ecology of the crayfish Cambarus immunis (Hagen). American Midland Naturalist. 25:420-444.

Taylor, R.C. 1984. Thermal preference and temporal distribution in 3 crayfish species. Comp. Biochem. Physiol. A Comp. Physiol. 77(3):513-518.

Thorp, J.H. 1978. Antagonistic behavior in crayfish in relation to temperature and reproduction. Oecologia. 36:273-280.

Vander, A.J, J.H. Sherman, and D.S. Luciano. 1980. Human physiology: the mechanisms of body function. McGraw-Hill, Inc. New York, NY.

White, R.L. 1983. Effects of acute temperature change and acclimation temperature on neuromuscular function and lethality in crayfish Procambarus darhi. Physiological Zoology. 56(2):174-194.

Weins, A.W., and K.B. Armitage. 1961. The oxygen consumption of Orconectes immunis and O. nais in response to temperature and to oxygen saturation. Physiological Zoology. 34:39-54.

Wetzel, R.G. 1983. Limnology, 2nd ed. Saunders College Publishing. Philadelphia, PA.

Williams, A.B., and A.B. Leonard. 1952. The crayfish of Kansas. University of Kansas Science Bulletin, Vol. XXXIV, part II, No. 15. p. 961-1012.

EVALUATING SURVIVAL OF THE CRAYFISH Orconectes nais EXPOSED TO HYPOXIC WINTER CONDITIONS

by

Charles Ephriam Kichler

B.S., Ohio State University, 1984

AN ABSTRACT OF A MASTER'S THESIS

Submitted in partial fulfillment of the
requirements for the degree

MASTER OF SCIENCE

Division of Biology

Kansas State University
Manhattan, Kansas

1987

ABSTRACT

Crayfish culture is a successful industry in the South. This industry was brought about as a direct consequence of an aggressive research program at southern universities. A potential exist for the development of a central states industry using the native crayfish Orconectes nais because of abundance and size.

A major problem that needs to be eliminated before culturing can be profitable is winter survival. A potential cause of winter kill is low dissolved oxygen (DO). This condition occurs ice and snow cover prevents oxygen diffusion and photosynthetic levels of light from entering a pond. In vivo studies were not possible; therefore, an in vitro method of study was developed to circumvent problems. The method consisted of a system of four chambers partially submerged in a cooling chamber. Each chamber had a closed water circulating system and an artificial atmosphere.

The survival of the crayfish O. nais was evaluated in two experiments under simulated winter conditions. The first used two winter temperatures and two low DO levels over 240 h. Greater than 50% survival was realized at both temperatures at 0.3 mg/L DO. The second evaluated survival rate at 4°C and 0.04 mg/L DO over time. Greater than 50%

survived for 3 d and almost none survived for 12 d. Both experimental results were used to develop a linear model of survival. Practical implications to crayfish aquaculture are discussed in the conclusion.