ASPECTS OF REPRODUCTION FOR THE CRAYFISH

Orconectes nais

by

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PREFACE

This study examines the reproductive activities of the crayfish *Orconectes nais* and is part of an ongoing study to determine the culture potential and possible methods of culture for this species. Reproduction is an integral part of all life and the knowledge of its details is essential to the raising of cultured animals.

Crayfish culture is a rapidly growing industry in the southern United States. Huner (1985) estimated production at 70-100 million pounds for *Procambarus clarkii* and *P. acutus acutus* with a retail value of $70 million for the United States in 1985. This crop was produced from 115,000 acres of water in five southern states. Production in 1980 was estimated at 23.9 million pounds and at 55 million pounds in 1982. The growth in crayfish culture can be associated with the increase in their use as sportfish bait and the growing popularity of Cajun cuisine. A mini-trend in Cajun/Creole cooking has created new markets for crayfish (Katz, 1987). The production of these animals is still limited mainly to the southern states. Only recently have attempts been made to culture crayfish in the central states, though several appropriately sized species suitable for culture reside in this area of the country (Huner & Avault, 1981). One of these species is *Orconectes nais*. Ingelin (1984), Kichler (1987), Jackson (1972), Pippet

(1977) and Rice (1973) have researched _Orconectes nais_. The majority of this work has been on defining the natural history of the organism and its survivability. A comprehensive study of the reproductive capabilities was therefore warranted.

The objectives of this study are to define the annual reproductive cycle of _O. nais_ and the way in which factors such as growth, density, condition, and relative productivity affect this cycle. The lack of information on central states crayfish is mainly due to the concentration of research on the southern species. The research on the southern species has been pivotal in the growth of crayfish culture in those states. A similar effort in the central states is needed to expand the current available knowledge for culture of the species present in this region of the country. This study represents part of a continuing research project to provide the necessary information for the culture of one of these species, _Orconectes nais_.

LITERATURE REVIEW

This thesis is concerned with aspects of the reproduction of *Orconectes nais*. The common crayfish, *O. nais*, belongs to the class Crustacea in the Phylum Arthropoda. The possibility of culturing this species has brought about a need for more detailed life history information than is currently available. Other studies on *O. nais* have been related to defining its general natural history and survivability. This study is necessary to provide a comprehensive qualitative and quantitative understanding of the reproduction of the species.

Crayfish are interesting animals both from an ecological perspective and an economic view. Crayfish have been shown to be polytrophic (Momot, 1978), able to withstand extremes of cold, and near hypoxic conditions (Kichler, 1987). The durability of these animals has enabled them to cover a wide geographical and ecological distribution. Crayfish lend themselves to polyculture with certain fish species and can be produced from otherwise unproductive waters (Huner and Dupree, 1984). The popularity of crayfish for both human consumption and as sportfish bait has raised interest in culturing various species of these animals commercially. One such species of interest is *O. nais*. Managing a population of these animals for production requires the understanding of the way they reproduce and the factors that influence reproduction.

Reproductive studies have been conducted for many species in the United States and Europe. One distinguishing feature of the crayfish, as a group, is the use of two different body forms by the male. The male crayfish when juvenile is in a non-reproductive form in which the first two pairs of swimmerets or gonopods, though dissimilar to the other swimmerets, are blunt and have a pronghorned-like appearance (Huxley, 1880). With maturation over several molts, the gonopods become more pointed with a dorsal channel. Through this channel sperm is transferred to the female during mating. After mating the male molts, resuming the non-reproductive form. This is called the second form or form II. The reproductive form is referred to as form I or the first form. The testis consist of three lobes located in the cephalothorax below and slightly anterior to the heart (Huxley, 1880). The testis produce the sperm which are stored in the vas deferens until expulsion from the male upon mating (Schramm, 1986). The testis then may regress (Smart, 1962).

The transfer of sperm to the female is accomplished through a complex mating ritual in which the male approaches the female, grasps either her pincers, rostrum or walking legs in his pincers and turns her over so that the female's ventral surface is facing the male's (Huner and Barr, 1984).

The male then secures himself to the female using a set of hooks located on the third segment of the third and fourth walking legs. The gonopods are then placed onto the annulus ventralis of the female. This is a "blind" pouch located between the fourth and fifth pairs of walking legs on the female. Before the sperm are actually deposited the annulus may be cleaned by the male (Andrews, 1904). The openings of the vas deferens are then extended into grooves on the dorsal side of the gonopods and the non-motile sperm released and channeled into the annulus. The deposited sperm form a small nodule in the female's annulus referred to as a "sperm plug". Several species of crayfish lack the annulus on the female, for example species in the genus Pacifastacus. In these species, the male places spermatophores, or sperm packets on the ventral surface of the female (Schramm, 1986). In either case, the sperm are stored externally until egg-laying at which time fertilization takes place.

The ovaries were found to enlarge greatly in several different species, but at different times of the year presumably dependent on seasonal variations in mating and egg-laying. Paranephrops planifrons, from New Zealand, Astacus astacus, a European species, and Pacifastacus leniusculus, common on the west coast of the U.S., reproduce continuously throughout the year (Momot, 1978). Procambarus *clarkii* mates in the spring and lays eggs throughout the summer and fall in burrows (Penn, 1943). This is typical of many of the southern species. Northern crayfish types tend to mate in the late winter and lay eggs in the spring (Weagle and Ozburn, 1971; Boyd and Page, 1978).

The act of egg-laying in crayfish is referred to as oviposition. The eggs are released from the oviducts which run from the ovary to an opening at the base of the second pair of walking legs (Huxley, 1880). Prior to oviposition the female vigorously cleans the underside of the abdomen and the pleopods with her walking legs (Mason, 1969). A mucous secretion called glair is then secreted from glands on the uropods (Andrews, 1904) and the abdomen is cupped to form a receptacle for the eggs. The eggs are released from the oviducts and moved along between the walking legs until they come to rest in the glair covering the tail. The number of eggs laid by any one female is strongly correlated with carapace length for most species with larger females producing more eggs (Penn, 1943; Smart, 1962; Payne, 1971; Boyd and Page, 1978; Huner and Barr, 1984). Carapace length is defined as the measurement from the tip of the rostrum to the point where the cephalothorax meets the abdomen and is usually expressed in either millimeters or inches. This measurement is the most commonly used body size measurement for crayfish and is equal to approximately half the total body length. Minimum mating sizes in the form of carapace lengths have been reported for several species; 22 mm for Orconectes immunis (Tack, 1941), 31 mm for Procambarus clarkii (Penn, 1943), 18 mm for Cambarus longulus longulus (Smart, 1962), and 18 mm for Orconectes palmeri palmeri (Payne and Price, 1981). The two or three year lifespans of these animals reduce the maximum attainable size and the maximum egg production of a female. Females carrying eggs become secretive and aggressive and either retire to burrows or find some structure in or near which to hide (Mason, 1969; Huner and Barr, 1984). The eggs are fanned by movements of the pleopods to allow oxygenated water to reach the developing embryos. The eggs' development is referred to as direct development (Williamson, 1982), in that the young appear nearly fully formed upon hatching.

Temperature affects hatching success and length of incubation. Rhodes (1981) discovered that the eggs of Austropotamobius pallipes when removed from the female could be hatched artificially and developed more rapidly when placed at higher than ambient temperatures. The eggs required a rise in temperature to hatch and deformities occurred at excessively high temperatures. Individual broods of Pacifastacus trowbridgi hatch synchronously and the young remain attached to the mother's pleopods with their pincers (Mason, 1970). During this period the young crayfish undergo two molts. At the end of this period they appear as fully formed miniature adults and leave the parent. After mating and rearing of the young, females undergo a post reproductive molt (Huner and Barr, 1984). The males molt after mating, resuming the non-reproductive form II until the next mating season (Huner and Barr, 1984). Geographic location and climate appear to have high impacts on time of mating and egg-laying. Southern types tend to mate in the spring and lay eggs in the summer or fall. Northern crayfish types tend to mate in the fall or late winter and lay eggs in the spring. Both northern and southern variaties are found in the genus Orconectes.

Orconectes clypeatus is common in Louisiana and is representative of the southern members of the genus. Female O. clypeatus retire to burrows to lay their eggs in the early fall (Smith, 1953). The young hatch from October to possibly as late as December. The exact period of mating is not known, but is presumed to begin in August and may last until December. The first young appear in October and newly-hatched young have been seen as late as December. The burrowing habits of the female may be in response to summer dessication of the ponds and marshes in which this species lives. This may not be a purely environmentally induced response since the females burrow even when water is available (Smith, 1953).

Orconectes virilis is a northern species in the genus. The mating season for O. virilis in northwestern Ontario was August through September (Weagle and Ozburn,1971). The females then overwinter in rivers, ponds and lakes laying their eggs in late May and June. The overwintering of females appears to be necessary for proper ovarian development. Aiken (1969) found that female O. virilis required constant darkness and low temperature for 4-5 months to complete ovary maturation. The eggs hatch in July and approximately half of the young reach maturity in the first year. In contrast to these findings, Berrill and Arsenault (1982) found that Orconectes rusticus , studied in southern Ontario, mated in the spring before egg laying. These spring mating periods are of short duration (about four weeks) and may be cued environmentally by water temperature.

Central states species offer a different pattern. Boyd and Page (1978) found that Orconectes kentuckiensis in Illinois mated in October and November with oviposition occuring in March, April and May. Andrews (1904) observed mating in February and March in Orconectes limosus collected in Maryland. Fall matings were also seen in this study further confusing the issue of time of mating. Egg laying does appear to be restricted in the northern types to March through May.

The particular reproductive cycle of Orconectes nais has recieved only a small amount of attention. Jackson (1972) offered only a brief account of this species. Ingelin (1984) presented some evidence to support the belief of Williams and Leonard (1952) that this species mates in the late summer and fall with eggs laid in the spring. Hatching was suggested to occur in May as young crayfish were recovered in June samples. Ingelin also noted that form I males first appeared in June and increased in abundance through November.

The length of time required for crayfish to reach maturity is dependent on several factors. Minimum sizes for egg production in females would suggest that growth is an important regulator of reproduction (Huner and Barr,1984). Young-of-the-year stunted by overcrowding may not reach mating size by the end of the first growing season (Jackson, 1972). Density, food supply, water temperature, dissolved oxygen content, and growing season are some of the factors that regulate growth (Huner and Romaire,1978; Ingelin, 1984) and, therefore, reproduction.

ASPECTS OF REPRODUCTION FOR THE CRAYFISH

Orconectes nais

INTRODUCTION

This study examines the reproduction of the crayfish *Orconectes nais*. The reproductive cycles of several species have been investigated. This species has recieved little attention outside of physiological and behavioral studies (Weins and Armitage, 1961; Pippet, 1977; Armitage and Wall, 1982). The recent interest in culturing *O. nais* for human consumption and sportfish bait has made such a study necessary. An understanding of the reproduction of this species is essential to its successful culture.

This study was conducted in three parts presented in the form of three chapters. Chapter 1 defines the overall reproductive cycle, chapter 2 is concerned with the effect of temperature on the incubation and hatching of the eggs, and chapter 3 examines the effects of habitat productivity levels on the reproductive cycle of *O. nais*.

Yearly reproductive cycles for several species have been described. The application of these studies to *O. nais* is questionable due to geographic and habitat differences. Few studies are available on pond dwelling species in the central states (Williams and Leonard, 1952; Jackson, 1972; Ingelin, 1984). These studies propose that for *Orconectes* nais mating occurs in the fall and egg-laying in the spring, with hatching occuring in May. Incubation studies are also not commonplace. The effects of temperature on incubation was examined by Rhodes (1981) on the British species Austropotamobius pallipes and by Aiken (1969) on ovary maturation and hatching for Orconectes virilis, a northern species. The general findings of these studies suggest that higher than natural temperatures cause a reduction in the incubation period. Rhodes (1981) did note that at temperatures much greater than found in nature, deformities and improper hatching increased mortality among newly-hatched crayfish.

The effects of poor habitat quality on reproduction have gone largely unexplored. Growth in relation to nutrition and differences in population density have been researched extensively for Orconectes virilis (Momot, 1978), Procamburus clarkii (Huner and Barr, 1984) and Orconectes nais (Ingelin, 1984). Momot (1978) found that mating success was inversely related to density as was survival of the young crayfish. This is the only study known to the author that addresses this aspect of reproductive success in response to density for crayfish. This study was undertaken to describe the reproductive cycle, incubation period in relation to temperature, and effect of habitat productivity on reproductive success for Orconectes nais.

CHAPTER I

THE SEASONAL REPRODUCTIVE CYCLE OF

Orconectes nais

Materials and Methods

One pond was used in this study and will be referred to as pond 1. Pond 1 has been noted in past years for its high production of crayfish yielding an average of 809 kg/ha (722 lbs/acre) over the past three years (Unpublished data). This pond has a total area when full of 0.162 ha (0.40 acre), a total volume of 1,431 cubic meters (1.16 acre ft), and a maximum depth of 1.98 m (6.5 ft). The average area during the growing season of 1987 was 0.155 ha (0.382 acre). Cattle were present in the pasture surrounding pond 1 and were frequently seen wading in the pond during the study period. Throughout most of the year the pond was slightly turbid (0.2 to 0.9 Sechi disk depth) and received a small amount of input from a spring in addition to runoff from the surrounding pasture. Aquatic vegetation was absent at all times during the study period in pond 1. Pond 1 is located on Kansas State University Pastures in Riley County, Kansas.

Crayfish were seined periodically from May 1987 through April 1988 from Pond 1 with a 6 m (20 ft) by 1.2 m (4 ft) bag seine of 6.35 mm (1/4 in) mesh. Each collection consisted of 25 males and 25 females of each year class present in the pond, when possible. Adults and young-of-the-year (YOY) were separated by relative size, with the larger animals being considered adults. The management regime for the pond involved heavy harvest of crayfish during the study period which resulted in the elimination of the adults by the end of August 1987. Collections were made every three to four weeks with the exception of the period lasting from the middle of December 1987 to late February 1988. During this period collecting was suspended due to ice cover on the ponds and poor field conditions. Both YOY and adults were taken when present. The crayfish were then transported to the laboratory and preserved in a buffered 10% formalin solution until processed.

The numbers of males, females, and egg-bearing females were recorded for each date. Carapace length and wet body weight was recorded for each specimen. Carapace length is the measurement from the tip of the rostrum to the point where the cephalothorax meets the abdomen. This is commonly expressed in millimeters and is used as a measure of body size. Wet body weight was measured by allowing the crayfish to drip dry before weighing. The carapace was then cut away and the gonads removed. Ovaries were weighed and examined for development. Ovarian egg diameters were measured under a dissecting microscope equipped with a graduated scale in the eyepiece. Ten eggs were measured at random from each female crayfish. Reproductive form was recorded for each male crayfish. Testis were removed and weighed. The gonads were then replaced in the body cavity and the animals dried for 5 days at 55-60°C. Utilizing the wet body weight and gonad weight a Gonadosomatic Index (GSI) was calculated for each animal. The GSI is a relative measure of the weight of an animal's gonads expressed as a percentage of the animal's body weight. The formula used is as follows:

$$GSI = (\text{Gonad weight} / \text{Body weight}) \times 100$$

where GSI = Gonadosomatic index.

The dry weight measure in conjunction with wet body weight was used to calculate an Internal Condition Index (ICI) for each crayfish. The ICI measures the displacement of lost tissue inside the shell by water, which is then driven out by drying. The weight of the dried tissue remaining is then compared to the wet body weight recorded before drying for the animal. The formula:

$$ICI = (\text{Dry body weight} / \text{Wet body weight}) \times 100$$

gives a measure of the percent of tissue present in the animal that is not water. In a preliminary experiment, the ICI's were determined for starved and non-starved crayfish over varying periods of time. The ICI's of the starved animals decreased over time, but their wet weight did not change. The ICI's of the non-starved animals did not change over time. Wet weight was also constant for the non-starved animals over time. The catabolism of internal tissues in the form of food reserves during starvation yields a lower dry weight. The wet weight does not change measurably due to the displacement of these tissues by an equal volume of water. Dividing the dry body weight by the wet weight of the animal gives a relative measure of the internal tissue present in the animal. This gives an accurate reflection of the internal condition of the animal.

Statistical comparisons were performed using a least significant difference multiple comparison analysis with alpha=0.05. The SAS statistical analysis package (version 5.16) at the Kansas State University Computing Center was used for these statistical calculations.

Results and Discussion

Figure 1 shows the seasonal testes development in the form of average GSI of the male crayfish in pond 1 from May 1987 to April 1988. The GSI for the adult male crayfish collected from May 1987 through July 1987 (Figure 1) showed no significant change (p=0.05). Young-of-the-year (YOY) males collected from July 1987 through August 1987 showed a significant increase in their Gonadosomatic Index (GSI) (Figure 1), reaching a peak of 0.378 for the late August

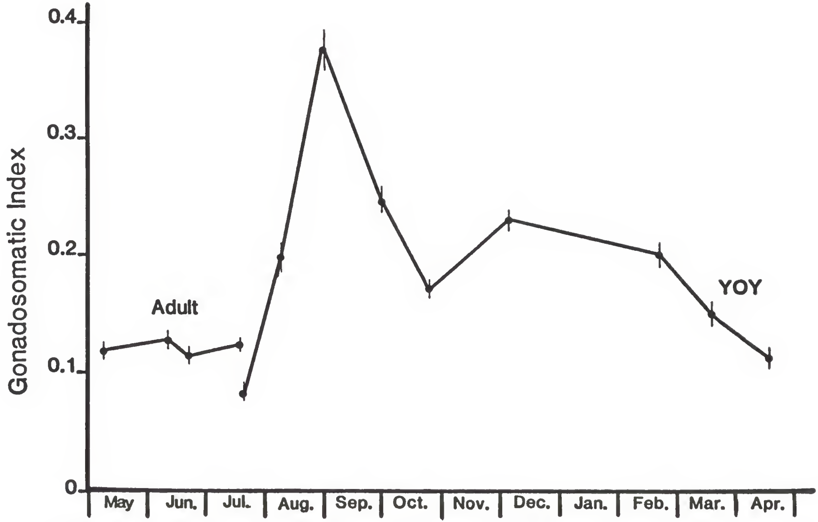

Figure 1. Plot of the Gonadosomatic Index for the male crayfish in pond 1.
*Bars represent the mean +/- one SD from the mean.

1987 collection (p=0.05). YOY male crayfish collected between September 1987 and December 1987 did not have any significant change in their GSI (p=0.05). YOY male crayfish collected from February 1988 through April 1988 showed a significant decrease in their GSI (Figure 1) (p=0.05).

Figure 2 demonstrates the seasonal changes in condition in the form of the ICI for the male crayfish in pond 1. The ICI for the YOY males did not change significantly from the July 1987 collection through the September 1987 collection (Figure 2) (p=0.05). A significant increase did occur from the September 1987 collection through the February 1988 collection (p=0.05). From the February 1988 collection through the April 1988 collection the ICI decreased significantly (Figure 2) (p=0.05).

Table 1 presents the percentage of form I adult males collected at each collecting date. Adult form I males began to appear in the June 1987 collection and increased in percentage of the population through the July 1987 collection (Table 1). Form I YOY males were first collected in September 1987 (Table 2). By late September 96% of the YOY males collected were form I. This increased to 100% by the December 1987 collection. The percentage of form I males in the population decreased from the February 1988 collection through the April 1988 collection (Table 2), at which point only 4% of the males collected were form I.

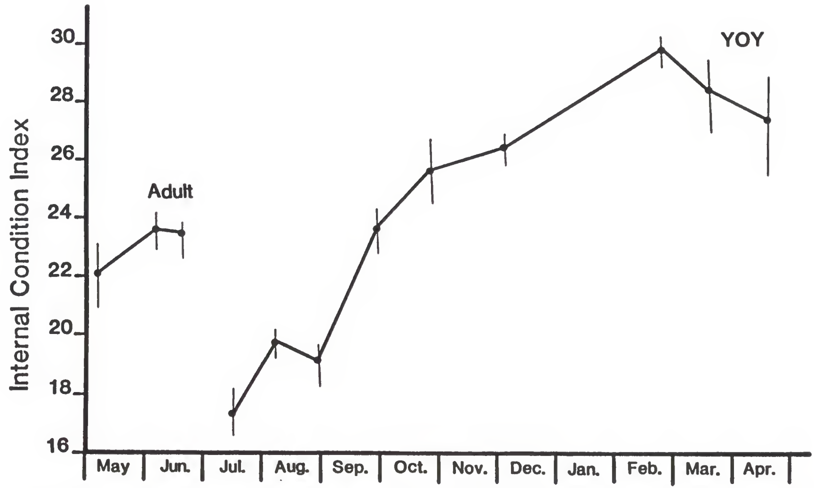

Figure 2. Plot of the Internal Condition Index for the male crayfish in pond 1. *Bars represent the mean +/- one SD from the mean.

Table 1. Percentage of form I adult males collected in pond 1.

Date	Number Form I	Number Form II	Total	Percent Form I
May 8	0	25	25	0
June 10	10	15	25	40
June 25	18	7	25	72
July 21	6	4	10	60

Table 2. Percentage of form I YOY males collected in pond 1.

Date	Number Form I	Number Form II	Total	Percent Form I
July 21	0	25	25	0
Aug 11	0	25	25	0
Sept 1	2	23	25	8
Sept 30	24	1	25	96
Oct 28	24	1	25	96
Dec 5	25	0	25	100
Feb 25	23	2	25	92
March 23	19	6	25	76
April 20	1	24	25	4

The peak of the YOY males' GSI (Figure 1) in August would suggest that sperm production also peaks at that time. This is also the period when the majority of the males molted into the form I condition (Table 2). Fertilization could take place at any point after that time. Sperm plugs were not evident on any of the females collected in this study. Exact time of sperm deposit is not known, though mating was observed in aquaria on frequent occasions from summer to spring. Females with white annulae that appeared to have been cleaned were also seen during the late fall and spring. Andrews (1904) suggested that the males cleaned the annulus as part of the mating ritual prior to deposition of sperm. Schram (1986) found that sperm were stored in the vas deferens after being produced in the testis. The fact that only the testes were weighed to formulate the GSI measurement for each individual, would indicate that the GSI is only valid for development of the testes. Storage of sperm in the vas deferens could occur without a noticeable difference in the GSI recorded. The GSI, therefore, reflects sperm production and not storage (Figure 1).

The ICI undergoes a drastic increase immediately after the GSI peak in August (Figure 2). This could demonstrate a shift in energy priorities from reproduction and growth to food storage for winter survival. Dormancy in winter limits the animals' ability to forage (Schram, 1986). Producing the energy rich sperm before this period would reduce energy requirements over the winter and enhance survival. The continual increase in the ICI (Figure 2) over the winter may be due to death of the animals with lower ICI values, skewing the collected values. The abrupt decline in March and April is assumed to be due to the use of stored energy in mating. At this time, the majority of the males resume the form II condition (Table 2). Mating would, therefore, need to occur between September and April.

Figure 3 shows the seasonal changes in the state of the female crayfish' ovaries in pond 1 from May 1987 to April 1988. The Gonadosomatic Index for adult females (Figure 3) collected from May 1987 through July 1987 showed no significant change (p=0.05). This is assumed to be the post spawning resting period. A significant increase in the GSI for the adult females was recorded from July 1987 through September 1987. Young-of-the-year females collected from July 1987 through February 1988 showed a continual increase in their GSI (Figure 3). The YOY females' GSI decreased from February 1988 through April 1988. This coincides with the occurence of egg-bearing females in the collections. Egg-bearing females were first collected in March 1988 (Table 3). 33.8% of the females collected were carrying eggs on this date. This percentage increased to 77.9% for early April before decreasing through the end of April and

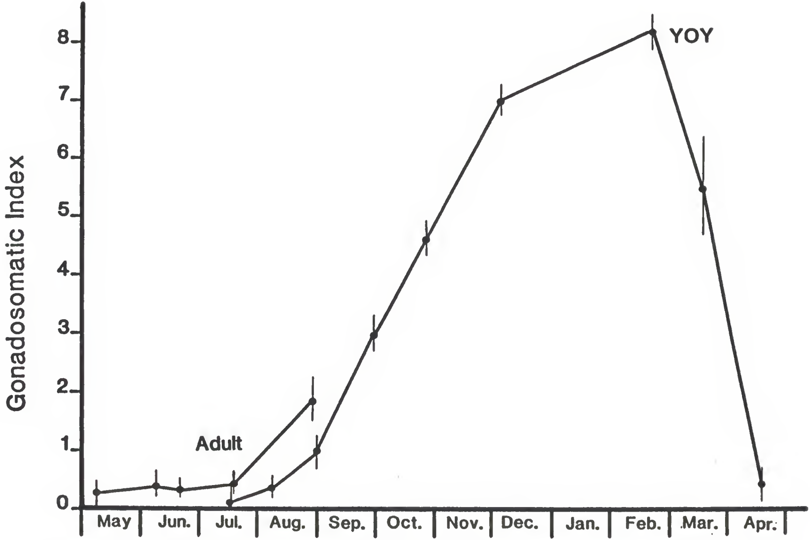

Figure 3. Plot of the Gonadosomatic Index for the female crayfish in pond 1.
*Bars represent the mean +/- one SD from the mean.

Table 3. Percentage females bearing eggs in pond 1.

Date	No. with eggs	No. without eggs	Total	Percent with eggs
Feb 25	0	25	25	0
March 23	24	47	71	33.8
April 7	81	23	104	77.9
April 20	8	14	22	36.4
May 12	8	42	50	16.0

early May to a value of 16% (Table 3). The number of eggs carried by a female varied, even among those of similar size. Dead, or fungused eggs were also evident on many of the females collected , having an orange or yellow appearance rather than the slate grey color normally seen.

Mean ovarian egg diameters for adult females (Figure 4) increased significantly from July 1987 through September 1987 ($p=0.05$). Mean egg diameters for YOY females increased from July 1987 through February 1988 ($p=0.05$). Mean egg diameter for the YOY females remained fairly constant through March and decreased through April (Figure 4).

The Internal Condition Index for the adult females (Figure 5) showed no significant change over any of the collections ($p=0.05$). The ICI for the YOY females (Figure 5) increased significantly from July 1987 through February 1988 ($p=0.05$). The ICI then decreased through March and April.

Ovary growth in the female crayfish peaked at the end of February 1988 (Figure 3). At this time, egg-laying commenced and continued throughout April (Table 3). Egg diameter increased throughout the fall, but was nearly unchanged over the winter months (Figure 4). The smallest increases in egg diameter and GSI occur at the time of year when water temperatures are the lowest. As the temperature

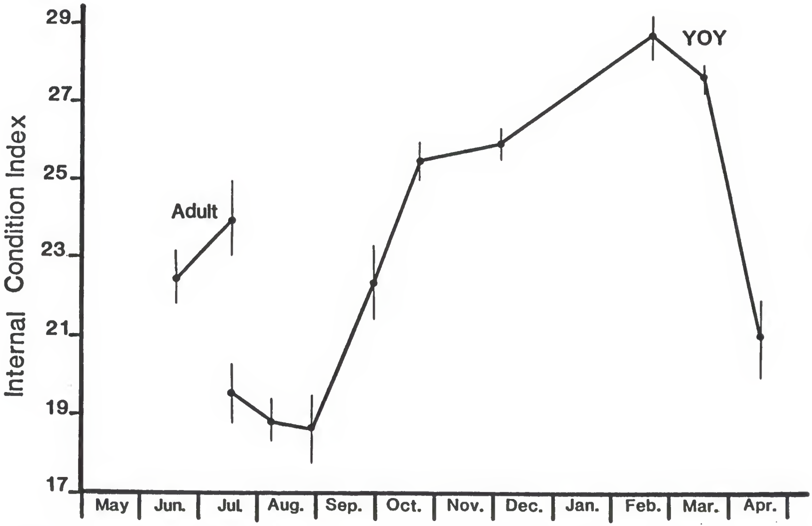

Figure 4. Plot of the Internal Condition Index for the female crayfish in pond 1. *Bars represent the mean +/- one SD from the mean.

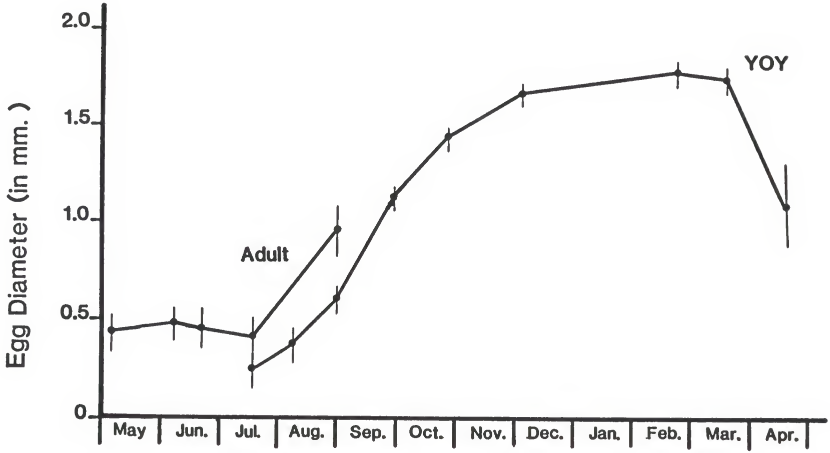

Figure 5. Plot of the mean ovarian egg diameters for the female crayfish in pond 1. *Bars represent the mean +/- one SD from the mean.

began to increase, egg-laying began. Aiken (1969) found overwintering to be vital for ovarian development in Orconectes virilis. The data collected in this study do not suggest that a similar relationship between winter conditions and ovarian development exists for Orconectes nais, since near maximum egg diameter is reached by early winter.

Energy would appear to be channeled into reproduction before the onset of winter. Egg development and sperm production are heaviest in the late summer and fall with a gradual reduction throughout the fall and winter. Storage of reserves for winter is reflected by the males ICI (Figure 2). The females' ICI (Figure 5) is more dependent on the development of the ovary which makes up a large amount of the animal's internal tissues. Winter survival of females would appear to be less likely than that of males due to the demands for energy and internal space for egg production.

CHAPTER II

EGG INCUBATION AND HATCHING IN *Orconectes nais*

Materials and Methods

Female crayfish were collected from Pond 1 (Chapter 1) on February 25, March 17, March 23, and April 20, 1988. All of the crayfish collected on the first two dates were believed to be young-of-the-year from 1987 and did not show signs of previous oviposition. Crayfish collected on the last two dates included both barren and egg-bearing females.

Six 76 liter (20 gal) glass aquaria equipped with fifteen 12.5 cm by 10 cm by 30 cm deep cages of 6.35 mm (1/4 in) mesh hail screen were set-up prior to the collections. Each tank had a continuous flow of approximately 5 liters per hour, and was aerated. Three temperature treatments were assigned to two tanks each. These were 8°C, 12°C or 16°C, respectively. This yielded 6 treatment tanks with two at each of the 3 temperatures. The bottom of each cage was covered with approximately a 2-3 cm layer of gravel. The tanks were exposed to diffused daylight in order to allow the animals to maintain their photoperiodic orientation.

There were 36 crayfish collected on each of the first two collecting dates, February 25 and March 17, 1988. Each collection of 36 was then randomly divided into 6 subgroups of 6 crayfish each. Each subgroup was then placed randomly in one of the six treatment tanks, one subgroup per tank from each collection. This gave a total of 6 treatment tanks each with 12 crayfish consisting of 6 crayfish from each collection date. This yielded 24 crayfish at each of the temperatures, or 12 per tank. The crayfish were fed pieces of fresh male crayfish twice a week. Date of egg-laying was then recorded for each female. Date of hatching of the young was also recorded. Throughout the incubation period, the crayfish were inspected for eggs or young and notes on general condition were taken at noon and dusk daily.

The crayfish collected on March 23, 1988 were sorted into egg-bearing and barren females. The 25 egg-bearing females' carapace lengths were recorded and the number of eggs laid counted. The females' ovaries were then removed and examined for developed eggs. Eight egg-bearing female crayfish procured on April 20, 1988 were placed in individual 3.25 mm wire mesh cages suspended in a fiberglass tank with a continuous flow of fresh water at 15°C. The animals were then inspected daily for hatched young. The number of females hatching eggs and incubation time since the collecting date were recorded.

Results and Discussion

The crayfish placed in the aquaria and held at either 8°C, 12°C, or 16°C appeared in good health throughout the experiment and no deaths were observed in any of the tanks. At 8°C 16 females out of 24 laid eggs though none of the eggs laid were successfully hatched (Table 4). Several of the females had dead eggs present after 10 days, but these were not removed by the females.

In the 12°C tanks 16 out of 24 female crayfish laid eggs and 3 crayfish successfully hatched their broods (Table 4). Average hatching time was 62.3 days with values of 62, 62, and 63 days being recorded for each of the 3 females, respectively. At 16°C 17 out of the 24 females laid eggs and 4 crayfish successfully hatched their broods in 33, 34, 35, and 35 days, respectively, with an average hatching time of 34.25 days (Table 4). Dead eggs were also present on the majority of the females maintained at 12°C and 16°C. The female crayfish were never observed removing these eggs. Dead eggs were common at all 3 temperatures. The exact cause of mortality was not determined for these eggs.

The egg-bearing females collected on March 23, 1988 yielded egg counts between 67 and 671 eggs (Figure 6). The number of eggs was regressed against carapace length and found to be strongly related (R^2 = 0.8291). The linear

Table 4. Percent of female crayfish hatching eggs that laid eggs and hatching time in days at three temperatures.

Temperature (in °C)	No. Laid	No. Hatched	Percent Hatched	Hatching Time (in days)
8	16	0	0.0	-----
12	16	3	18.75	62.30
16	17	4	23.53	34.25

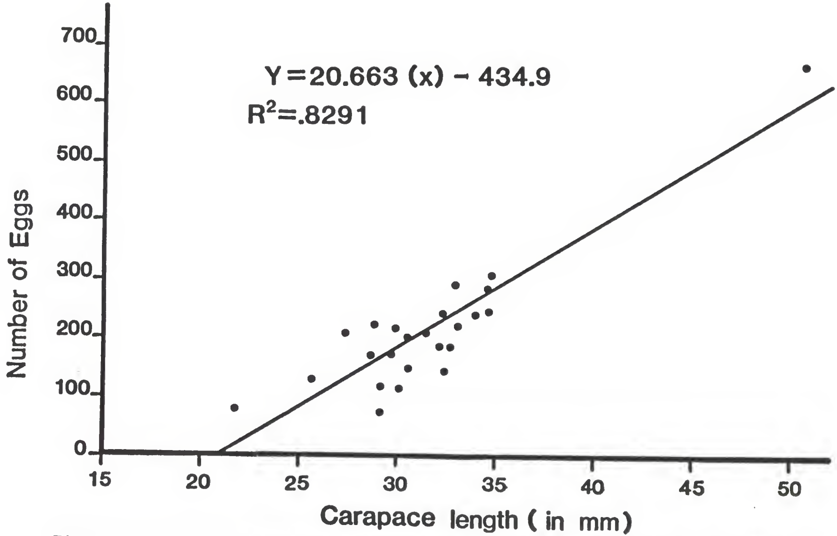

Figure 6. Plot of regression, carapace length vs. number of abdominal eggs.

equation:

$$Y = 20.663(X) - 434.899$$

provided the best fit, where Y = number of eggs carried and X = carapace length in mm (measured from the tip of the rostrum to the point where the cephalothorax meets the abdomen). A minimum size for egg production was obtained from this equation by setting Y = 0 and solving for X. A carapace length of 21.0 mm would be the minimum size for egg production determined from this equation. The value of 671 eggs was recorded for what was believed to be a second year adult female. Removal of this point from the data did not significantly change the regression coefficient or the regression equation. The number of eggs a female lays, therefore, is determined by the female's body size to a large extent. Generally the larger the female the more eggs that will be laid. Examination of the ovaries of egg bearing females showed no remaining eggs in the ovary. Egg-laying, would therefore, appear to be limited to one batch per year.

The poor hatching noted in the tanks maintained at 8°C, 12°C, and 16°C could be due to infertility of the eggs, not an inherent flaw in the experimental set-up. The fact that all six tanks experienced a similar problem with large numbers of dead eggs would indicate that some factor inherent to the crayfish was responsible. If fall mating occured, then a greater hatching success should have been observed. The fact that some of the eggs did hatch means that some of the females must have had viable sperm present in their annulae, suggesting that mating may need to occur within a short period before egg-laying to insure fertilization of the eggs or that sperm only remain viable for a relatively short period of time after being deposited in the female's annulus.

The 8 egg-bearing females collected on April 20, 1988 all successfully hatched their broods. The 8 females were obviously fertile when removed from the pond. Incubation time before hatching varied from 16 to 23 days after collection. All of the females hatching offers a sharp contrast to the females held in the aquaria from the February 25 and March 17, 1988 collections, of which only a few managed to hatch their broods. This supports the previous arguments that mating occurs shortly before egg-laying, or that the viability of the sperm is of a short duration requiring mating shortly before egg-laying to insure fertilization. Effective fall mating does not seem a likely possibility in light of these results.

CHAPTER III

THE INFLUENCE OF HABITAT PRODUCTIVITY ON THE REPRODUCTION OF *Orconectes nais*

Materials and Methods

Two ponds were used in this study. Pond 1 has been noted in past years for its high production of crayfish yielding an average of 809 kg/ha (722 lbs/ac) over the past three years (Unpublished data). Located on Kansas State University Pastures this pond was also used for the two previous experiments. Pond 1 has a total area when full of 0.162 ha (0.40 acre), a total volume of 1,431 cubic meters (1.16 acre ft), and a maximum depth of 1.98 m (6.5 ft). The average area during the growing season of 1987 was 0.155 ha (0.382 acre). Cattle were present in the pasture surrounding the pond and were frequently seen wading in the pond during the study period. Through most of the year it was slightly turbid (0.2 to 0.9 m Sechi disk depth). and recieved a small amount of input from a spring in addition to runoff from the surrounding pasture. Aquatic vegetation was absent at all times throughout the year in this pond.

Pond 2 drained an ungrazed pasture and in recent years had lower yields of crayfish than pond 1 with an average yearly harvest of 237 kg/ha (234 lbs/ac) over the past three years (Unpublished data). Pond 2 has a total area when full of 0.232 ha (0.574 acre), a total volume of 2,195 cubic meters (1.78 acre ft), and a maximum depth of 2.13 m (7.0 ft). The average area during the growing season of 1987 was 0.201 ha (0.497 acre). Cattle were absent from the surrounding pasture during the study period. Pond 2 was slightly turbid throughout the study period (0.2 to 0.6 m Sechi disk depth). The only water source for this pond was runoff from the surrounding pasture. Trees were present on the dam of pond 2. Aquatic vegetation was absent throughout the study period in this pond. Both ponds were free of predatory fish. Both ponds are located in Riley County, Kansas.

Crayfish were seined from May 1987 to April 1988 at various time increments from both ponds with a 6 m (20 ft) by 1.2 m (4 ft) bag seine of 6.35 mm (1/4 in) mesh. Collections consisted of 25 males and 25 females of each year class present in each pond when possible. The management regime for both ponds involved heavy harvest of crayfish during the study period which resulted in the elimination of the adults in both ponds by the middle of September 1987. The crayfish were preserved in a 10% buffered formalin solution until processed. The carapace length, wet body weight and gonad weight was measured for each crayfish using the same procedure as detailed in

Chapter 1. Reproductive form was determined for males by inspection of the gonopods. Ovarian egg diameter was determined for females using a sample of ten eggs for each female and notes on ovary development recorded. A Gonadosomatic Index (GSI) and Internal Condition Index (ICI) was calculated for each crayfish using the procedures outlined in Chapter 1. The YOY crayfish in pond 2 were very small and reliable readings for the calculation of their GSI's and ICI's were not feasible until October of 1987 when they were of adequate size.

Population density was determined for each pond in September of 1987 using the method described by Ingelin (1984). The basic method consisted of using 1 square meter open-ended box traps consisting of a frame constructed of 1.3 cm (1/2 inch) angle iron with 6.35 mm (1/4 inch) hardware cloth covering the sides. One trap was 1.03 m (3 ft 5 inches) tall and was used to sample depths of 1 m or less. The second trap was 1.35 m (4 ft 5 inches) tall and was used to sample depths over 1 m. The traps were carried to the desired depth above the water suspended between two people using poles to carry the trap like a stretcher. The trap was then quickly lowered and forced into the bottom. Crayfish caught in the trap were then removed with a dip net and counted. Three measurements were conducted at each of five depths for each pond. Depths of 1/4 m, 1/2 m, 3/4 m, 1 m, and 1-1/4 m were sampled. The 1/4 m, 3/4 m, and 1-1/4 m depths were sampled along three transects made across the pond, starting at shore and proceeding to the middle of each pond. Three separate transects were made in a similar manner for the 1/2 m and 1 m depths. This approach was used to avoid disturbing crayfish at each sample site by allowing for more space between samples.

Results and Discussion

Comparing the male Gonadosomatic Indices between pond 1 and pond 2 the plot for pond 2 roughly parallelled the plot for pond 1, but at a lower level (Figure 7). Male GSI's in both ponds appeared to peak before October and then decreased after February. A similar pattern occured in the Internal Condition Indices for the two ponds (Figure 8). Males in pond 2 had lower ICI's than in pond 1, with both populations increasing to a peak in February 1988, then decreasing through April, 1988. Percentage of form I males in pond 2 was consistently lower than in pond 1 on any given date (Table 5). The crayfish in pond 1 also had a greater average carapace length than crayfish in pond 2 (Figure 9).

Females collected from pond 2 had lower average GSI's than those collected from pond 1 (Figure 10). Both populations' GSIs peaked in February and then declined through April 1988. The ICI for the females in pond 2 was

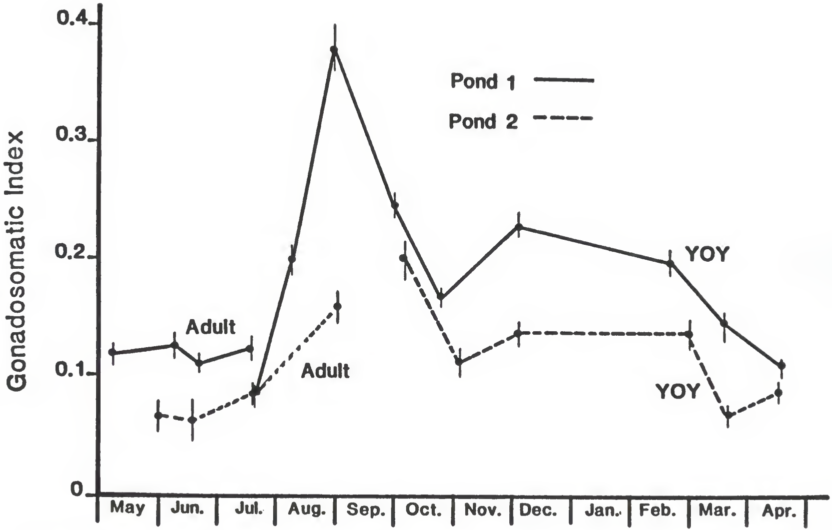

Figure 7. Plot of the Gonadosomatic Indices for male crayfish, pond 1 and pond 2.
*Bars represent the mean +/- one SD from the mean.

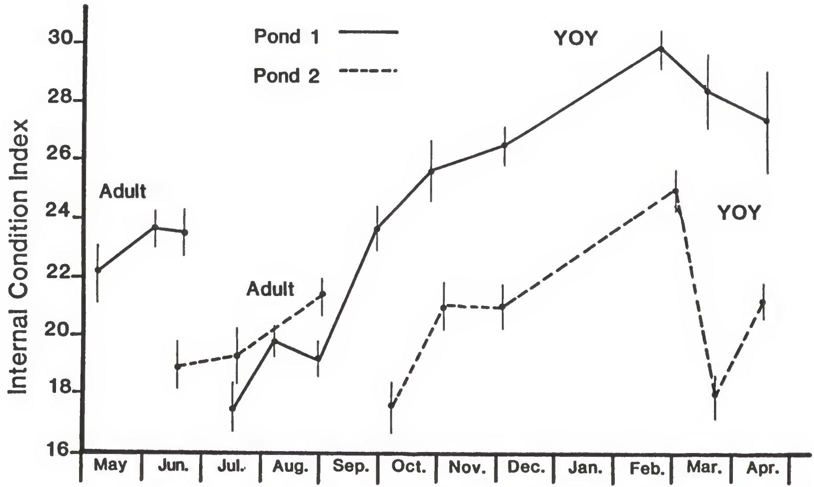

Figure 8. Plot of the Internal Condition Indices for the male crayfish, pond 1 and pond 2. *Bars represent the mean +/- one SD from the mean.

Table 5. Percentage form I males collected in pond 1 and pond 2 in 1987 and 1988.

Adults

	Pond 1			Pond 2	
Date	Percent Form I	N	Date	Percent Form I	N
May 8	0	25	----	--	--
June 10	40	25	June 1	0	24
June 25	72	25	June 22	32	25
July 21	60	10	July 22	36	25
----	--	--	Sept 2	46	13

YOY

	Pond 1			Pond 2	
Date	Percent Form I	N	Date	Percent Form I	N
July 21	0	25	July 22	0	94
Aug 11	0	25	Aug 10	0	107
Sept 1	8	25	Sept 2	0	100
Sept 30	96	25	Oct 7	68	25
Oct 28	96	25	Nov 4	60	25
Dec 5	100	25	Dec 3	40	25
Feb 25	92	25	March 1	72	25
March 23	76	25	March 24	28	25
April 20	4	25	April 19	4	25

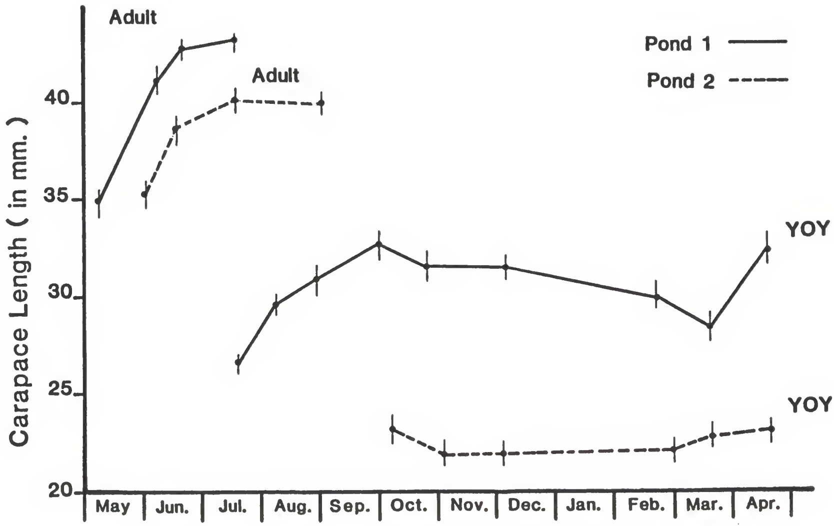

Figure 9. Plot of average carapace length for all crayfish, pond 1 and pond 2.
*Bars represent the mean +/- one SD from the mean.

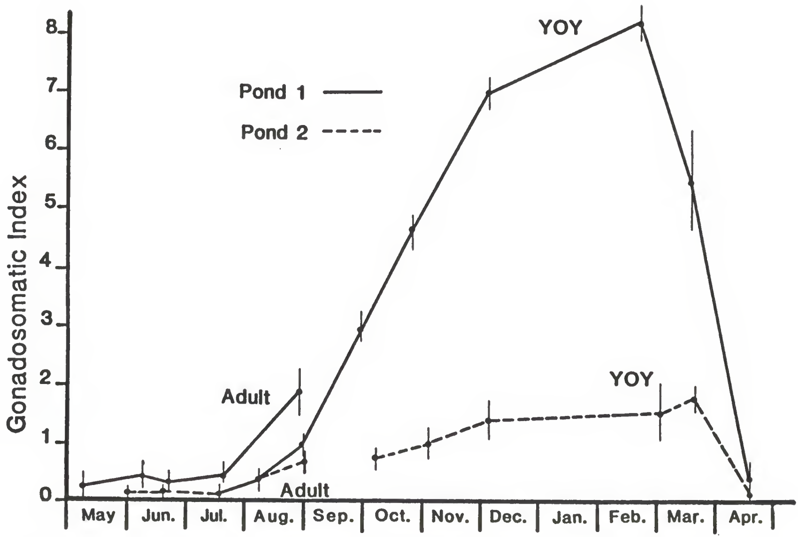

Figure 10. Plot of the Gonadosomatic Indices for the female crayfish, pond 1 and pond 2. *Bars represent the mean +/- one SD from the mean.

also lower than that for pond 1, with a peak during February 1988 (Figure 11). Mean egg diameter also peaked in February of 1988 for both ponds (Figure 12). The mean egg diameters in pond 2 females were smaller than those in pond 1 females. Fewer females in pond 2 had developed ovaries than in pond 1 (Table 6). Fewer females also laid eggs in pond 2 than in pond 1 from February to May 1988 (Table 7).

Density and distribution of the two populations differed greatly in September 1987 (Table 8). Pond 1 had an overall density of 4.8 crayfish per square meter. with the majority being found in depths of less than 5/8 meter. Pond 2 had an average density of 13.0 crayfish per square meter with a greater proportion of the population at depths greater than 5/8 meter.

In comparing the populations from pond 1 and pond 2, several differences are immediately apparent. Greater relative size of the gonads in both males (Figure 7) and females from pond 1 (Figure 10) would suggest that the reproductive output in pond 1 should be greater than in pond 2. Examining the relative numbers of females with developed ovaries (Table 6) and the number of egg-laying females (Table 7) this indeed is the case. Females in pond 1 were larger bodied (Figure 9), had larger ovaries containing larger eggs (Figure 12), and a higher ICI (Figure 11). Males in pond 1 managed to molt to the form I condition at a

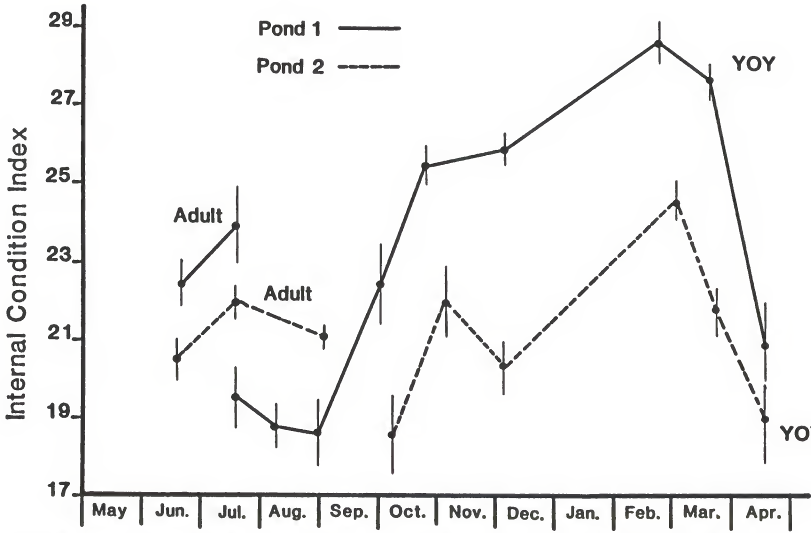

Figure 11. Plot of the Internal Condition Indices for the female crayfish, pond 1 and pond 2. *Bars represent the mean +/- one SD from the mean.

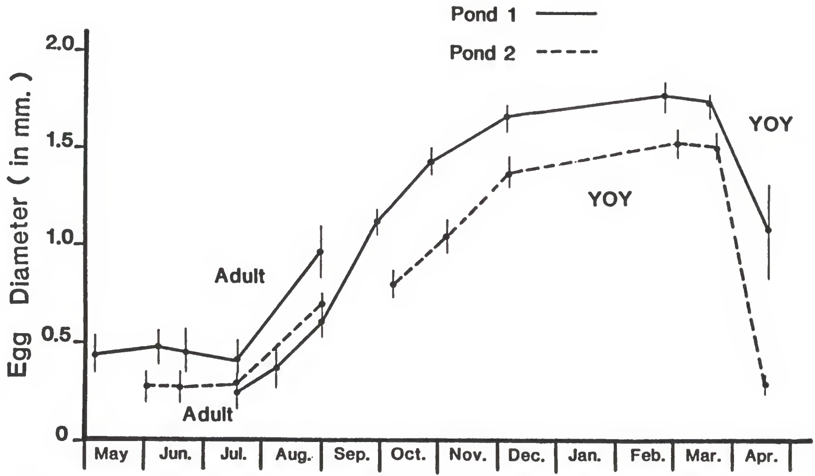

Figure 12. Plot of the mean ovarian egg diameters for the female crayfish, pond 1 and pond 2. *Bars represent the mean +/- one SD from the mean.

Table 6. Percentage of YOY females with developing ovaries.

	Pond 1			Pond 2	
Date	Percent Females	N	Date	Percent Females	N
July 21	96	25	----	--	--
Aug 11	100	25	----	--	--
Sept 1	100	25	----	--	--
Sept 30	94	24	Oct 7	52	25
Oct 28	96	25	Nov 4	36	23
Dec 5	96	25	Dec 3	40	25
Feb 25	100	25	March 1	36	25
March 23	27	35	March 24	4	25
April 20	23	13	April 19	4	25

Table 7. Percentage females bearing eggs in pond 1 and pond 2.

	Pond 1			Pond 2	
Date	Percent with eggs	N	Date	Percent with eggs	N
March 23	33.8	71	March 24	0.88	114
April 20	36.4	104	April 19	0.89	112

Table 8. Density and distribution for pond 1 and pond 2, September 1987.

Depth (m)	Pond 1 Crayfish/sq m	Pond 2 Crayfish/sq m
0-3/8	10.3	22.3
3/8-5/8	9.7	19.0
5/8-7/8	2.0	11.7
7/8-9/8	1.33	13.3
9/8-11/8	0.33	5.7
Weighted Average	4.8	13.0
Average Biomass (g/sq m)	51.7	42.5

higher rate than males in pond 2 (Table 5). Higher GSI's, ICI's, and greater body size affect reproductive output. The crayfish in pond 1 were able to attain reproductive status earlier and a greater part of the population was able to reproduce.

The greater density in numbers of crayfish in pond 2 could have caused the population to be stunted. The lack of cattle near pond 2 would lower the nutrient input of pond 2 when compared to pond 1, which would affect growth. This in turn would not allow individuals to reach a minimum mating and egg-laying size. The lack of form I males and large percent of females with undeveloped ovaries in pond 2 would support this conclusion. From the regression equation for carapace length and number of eggs laid (Chapter 2), a minimum size for egg production would be the x-intercept of 21.0 mm. The average carapace length of the population in pond 2 was 22.0 to 23.0 mm (Figure 9). Egg production was minimal (Table 7) and most likely due to the small size attained prior to the time of egg-laying. The crayfish in pond 1 had attained 26 mm in average carapace length before August of 1987 (Figure 9) and egg production was considerably greater than in pond 2 (Table 7).

Though density of individual crayfish is nearly three times as great in pond 2 biomass per square meter does not show as significant a difference (Table 8). The main effect on reproduction would appear to be density related not biomass related. Though pond 1 is much more productive, in terms of average harvest, the biomass present at a given time in either pond should be dictated by the carrying capacity of the pond. This capacity would appear to be greater in pond 1, but pond 2 contains greater numbers of crayfish. Density may reduce reproductive output in a crayfish population by increasing competition for resources and forcing weaker animals to inhabit less desirable areas of the pond. Energy for egg production and growth to a minimum mating size would be limited, allowing only a percentage of the population to reproduce. Holding the crayfish biomass below carrying capacity, for example by harvesting at a sufficient rate, might allow more individuals to become reproductively active.

CONCLUSION

The reproductive cycle of Orconectes nais begins in early summer when the male and female crayfish start developing gonads. Sperm production peaks in August or September and the sperm is then stored in the vas deferens. Egg production occurs, in the form of increased size of eggs not increased number, throughout the fall. During the winter months egg development slows, reaching a minimum when water temperatures are at their lowest.

Mating occurs throughout the fall and winter, but the habit of cleaning the annulus of the female prior to mating by the male, may only allow the most recent sperm deposited to fertilize the eggs. Mating also occurs in the spring in this species. It is believed that only these matings are responsible for the actual fertilization of the eggs. Fall matings would leave the sperm in the annulus ventralis for three to six months, where it is relatively unprotected. Spring matings could occur within weeks before egg-laying, insuring viability of the sperm and fertile eggs. In the spring, males are still in the reproductive form I while the majority of the females are laying eggs and mating at this time has been in observed in aquaria.

The production of the gametes before the onset of winter may be an adaptation for survival. The production of gametes before the onset of winter would allow for food reserves to be used for body maintenance, not reproductive effort during the winter, enhancing winter survival. Females have a decided disadvantage. They have a larger energy commitment toward egg production and less room for storage of food reserves than males due to the extensive size of the ovary in the late fall and winter. Females that don't lay eggs or have undeveloped ovaries may not have been able to procure enough energy to meet the demands of egg production and also be able to store adequate winter reserves.

The need to harvest crayfish for management reasons is clearly shown in this study. Allowing a population to be under harvested can result in stunted, nonreproductive animals, and a loss of control over such factors as desired size for marketing. Stocking densities for ponds in which reproduction is to be maximized need to be established for young and brood stock. Stocking at a proper density could reduce the need for such measures as supplemental feed for brood stock, increase number of young produced per female, and allow for grow out of young without cropping down a population, reducing the effort required to produce a crop. This study represents a first step in trying to answer a few of the many questions concerning reproduction in the crayfish, *Orconectes nais*.

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ASPECTS OF REPRODUCTION FOR THE CRAYFISH

Orconectes nais

by

Jonathan Howard Money

B.S. West Texas State University 1985

AN ABSTRACT OF A MASTER'S THESIS

Submitted in partial fullfillment of the

requirements for the degree

MASTER OF SCIENCE

Division of Biology

Kansas State University

Manhattan, Kansas

1988

ABSTRACT

This study examines the reproductive activities of the crayfish _Orconectes nais_ and is part of an ongoing study to determine the culture potential and possible methods of culture for this species. Reproduction is an integral part of all life and the knowledge of its details is essential to the raising of cultured animals.

The overall reproductive cycle was evaluated using a single pond and sampling the pond from May 1987 to April 1988. Gonad development was first observed in early summer in both male and female crayfish. Sperm production peaked at the end of August and the sperm is then believed to be stored in the vas deferens. The ovaries swell as egg production occurs, in the form of increased size of eggs not increased number, throughout the fall. During the winter months egg development slows, reaching a minimum when water temperatures are at their lowest. Mating occurs throughout the fall and winter, but the habit of cleaning the annulus of the female prior to mating by the male, may only allow the most recent sperm deposited to fertilize the eggs. Therefore, mating must also occur in the spring in this species. It is believed that only these matings are responsible for the actual fertilization of the eggs. The production of the gametes before the onset of winter may be an adaptation for survival. The production of gametes

before the onset of winter would allow for food reserves to be used for body maintenance, not reproductive effort during the winter, enhancing winter survival.

The linear relationship:

$$Y = 20.663(X) - 434.899$$

provided the best fit when the number of eggs oviposited by a female crayfish was regressed against carapace length. From this equation, a carapace length of 21.0 mm was determined to be the minimum size required for egg production. Crayfish eggs were exposed to temperatures of 8°C, 12°C, and 16°C. At the lowest temperature, none of the eggs hatched. Hatching time was found to be 62.3 days at 12°C and 34.25 days at 16°C.

Habitat productivity and its relationship to reproduction was investigated using a highly productive pond and a pond with lower yields of crayfish. The pond with lower production was found to have poorer reproduction, with late gonad development and a smaller percentage of the population reproducing than in the highly productive pond. The crayfish in the pond with lower production had a smaller average carapace length than the crayfish in the productive pond. Density was much greater in the lower production pond. Implications for crayfish aquaculture are discussed in the conclusion.