1995

Effects Of Diet And Environmental Factors On Growth, Survival And Physiology Of The Juvenile Crayfish, Cherax Quadricarinatus.

Mark Edward Meade
University of Alabama at Birmingham

EFFECTS OF DIET AND ENVIRONMENTAL FACTORS ON GROWTH, SURVIVAL AND PHYSIOLOGY OF THE JUVENILE CRAYFISH, *Cherax quadricarinatus*

by

MARK E. MEADE

A DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biology in the Graduate School, The University of Alabama at Birmingham

BIRMINGHAM, ALABAMA

1995

ABSTRACT OF DISSERTATION
GRADUATE SCHOOL, UNIVERSITY OF ALABAMA AT BIRMINGHAM

Degree PhD. Major Subject Biology

Name of Candidate Mark E. Meade

Title Effects of diet and environmental factors on growth, survival, and physiology of juvenile Australian crayfish, *Cherax quadricarinatus*

A series of experiments were performed to gather information that can be applied to developing efficient culture systems for the Australian crayfish, *Cherax quadricarinatus*. When juveniles were fed different formulated crustacean feeds, a wide range in performance, measured by weight gain and survival, was observed. A feed formulated at the University of Alabama at Birmingham (AB feed) yielded the highest weight gain and survival. Effects of the different feeds on growth patterns (molt intervals and molt increments) were specific, but could not be attributed to the lack of specific dietary nutrients. Juveniles have the ability to regulate oxygen consumption rates over a wide range of environmental oxygen tensions, and do not utilize anaerobic pathways to withstand hypoxic or anoxic conditions. Above or below a temperature range of 16 to 32°C, survival was low and weight gain was minimal. Highest weight gain and survival were observed at 28°C. Over a salinity range of 0 to 20 ppt, survival decreased above levels of 5 ppt and the highest weight gain was observed at salinities of 0 and 5 ppt. Metabolic rates were temperature dependent and energy utilization for growth was most efficient at a temperature of 24°C and at a salinity of 0 ppt. Metabolic rates were not affected by salinity nor acute exposure to ammonia or nitrate. Tolerances of juveniles to ammonia, nitrite, and nitrate are similar to those reported for other crayfish species. Nitrite substantially reduced the ability to regulate oxygen consumption rates. Overall, *C.*

quadricarinatus displays an ability to tolerate, grow, and survive at diverse environmental conditions, a good characteristic for its consideration as a candidate for commercial culture in many regions of the world.

Abstract Approved by: Committee Chairman _____

Program Director _____

Date 4/19/95 Dean of Graduate School _____

PS-5744

ACKNOWLEDGMENTS

The author wishes to thank all of those involved in the completion of this dissertation, including friends, faculty and graduate students in the Department of Biology. I would particularly like to thank my committee members, Dr. James McClintock, Dr. David Kraus, Dr. Louis D'Abramo, Dr. David Rouse, and Dr. Stephen Watts, for their invaluable input. I would further like to thank Dr. Jeannette Doeller for her aid in the interpretation of many data sets and Dr. Robert Angus for his aid in the statistical analyses of data. I would also like to thank Dr. Daniel Jones, Department Chairman, for his encouragement and financial support of this project. Special thanks also goes to Stacy James Stover. Lastly, I would like to thank my parents for their love and support for almost 30 years.

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GENERAL INTRODUCTION

Among the approximately 35,000 species of crustaceans, most occupy marine habitats, however, several species occupy freshwater or terrestrial habitats. Because of their diversity in aquatic habitats, crustaceans are considered not only of major importance to the ecosystem, but also of major economic value to man. Indeed, in many countries, commercial crustacean fisheries are an integral part of the economy (Neal and Maris, 1985; Haefner, Jr., 1985; Cobb and Wang, 1985). In the southeastern United States, crayfish culture comprises a major proportion of freshwater fisheries. For example, yearly production of crayfish in Louisiana in 1993 was estimated at 60-70,000 tonnes (Huner, 1994). As the number of culture facilities increases and culture methods are improved, production of crayfish in the United States is expected to reach 100,000 tonnes within the next decade.

The successful commercial culture of the red swamp crayfish, *Procambarus clarkii*, in the United States has prompted the import and evaluation of other crayfish species as prospective candidates for culture. Several Australian species have been examined for potential culture in the southeastern US (Semple, 1995). Among these, the Australian red claw crayfish, *Cherax quadricarinatus*, has demonstrated the greatest potential for culture, particularly because this species inhabits regions in Australia where the climate is similar to that in the southeastern US. Since the inception of commercial culture of the red claw in 1988, several investigators have reported encouraging production figures (Medley, 1994). At present, however, economists suggest that the production of this species in the US is cost prohibitive, principally due to high mortalities associated with juvenile culture and the subsequent low recovery of biomass (Medley, 1994). It has been suggested, however, that a better understanding of the biology of this

species will allow for improvements in management practices and resolve the problems associated with juvenile culture (Cobb and Wang, 1985).

Although some practical information concerning the culture of this species is available, information concerning the biology of the red claw relative to environmental conditions is not available. In Australia, *C. quadricarinatus* can be found inhabiting the northern regions surrounding the Gulf of Carpentaria (Reik, 1969). The climate in the coastal areas of northern Australia is generally more equable than the inland waters of Australia; nonetheless, *C. quadricarinatus* can be found inhabiting areas of diverse environmental condition. Water temperature, for example, can fluctuate substantially in many inland permanent streams. As temperatures increase during the summer months, many inland streams and ponds become dried. Crayfish inhabiting these regions excavate burrows searching for water in order to survive (Reik, 1969). When burrowing into the sediments, the crayfish can be exposed to reduced environmental oxygen tensions (McMahon, 1993; Reiber, 1993). Furthermore, many of the streams and ponds in Australia can be exposed to saline waters at various times during the year (Bayly, 1972). These reports strongly suggest that *C. quadricarinatus* is able to tolerate a wide range of diverse environmental conditions. The extent that different environmental conditions affect the growth and survival of *C. quadricarinatus* is unknown. Furthermore, the physiological and metabolic demands associated with the wide range of conditions previously mentioned have not been investigated.

Environmental conditions can greatly affect physiological processes (for review, see Vernberg, 1985). In order for an organism to survive, environmental factors must not exceed the tolerance limits where physiological functions are either compromised or ceased. A basic understanding of the relationships between environment and physiological processes is vital in assessing the ecological and commercial potential of a species. Often, the metabolic response of an organism can be used as an indicator of adaptability to different environments (Vernberg, 1985). Because *C. quadricarinatus* is adapted to

habitats characterized by diverse environmental conditions, it is an appropriate species to examine this relationship. Furthermore, because of the abrupt and sometimes drastic transitions imposed during early development, changes in environmental conditions can significantly affect the growth and physiology of larvae and juveniles (see Cameron and Mangum, 1985; Gilles and Pequeux, 1985; and Prosser and Heath, 1991, for reviews). No information exists concerning the effects of environmental factors on the growth and survival of juvenile *C. quadricarinatus*.

When examining the effects of environmental conditions on any species, healthy individuals must be used. Diets incomplete of specific nutritional components, however, can greatly affect the health of cultured crayfish (D'Abramo and Robinson, 1989). The nutritional requirements of *Cherax quadricarinatus* and many other crayfish species are not known. Confident determination of physiological responses of *C. quadricarinatus* to different environments requires a diet which promotes good growth and survival under optimal conditions. Furthermore, it has been suggested that defining a diet which promotes the growth and survival of crayfish will improve culture production (D'Abramo and Robinson, 1989). Current culture methods for *P. clarkii* involve use of forage based food systems. This practice consists of the infusion of agricultural crops, such as rice and sorghum, into ponds to provide a food source for crayfish consisting of detritus laden with microbes. Problems associated with food limitations and the accompanying stunting of populations in such settings, however, have prompted culturists to investigate for alternative culture methods. Use of formulated feeds has been suggested as an alternative method which, indeed, could be an effective method for enhancing growth in intensive cultures instead of blind reliance on non-specific sources of nutrition. Formulated feeds used in trial studies have proven successful in overcoming problems associated with food limitations in high density populations (for review, see D'Abramo and Robinson, 1989). Further studies, however, are needed to determine specific nutritional requirements of crayfish.

The objective of this study was to evaluate the growth, survival, and physiology of juvenile *C. quadricarinatus* (individuals ranging from 10-1000 mg wet weight) at different culture conditions. The following text consists of five articles. In the first article, weight gain and survival of juvenile crayfish cultured using formulated feeds were evaluated. In a second article, growth patterns (molt intervals and molt increments) of juvenile crayfish fed different diets were examined. In a third article, metabolic rates of juvenile crayfish exposed to varying environmental p_{O_2} were examined using respirometric and calorimetric techniques. In a fourth article, growth and survival, as well as growth efficiency, of juvenile crayfish exposed to different temperatures and salinities were examined. Finally, in a fifth article, crayfish survival and metabolism in relation to ammonia, nitrite or nitrate exposure were examined.

WEIGHT GAIN AND SURVIVAL OF JUVENILE AUSTRALIAN CRAYFISH, *Cherax quadricarinatus*, FED FORMULATED FEEDS

Authors: Mark E. Meade
Stephen A. Watts

Submitted as a note to: The Journal of the World Aquaculture Society

ABSTRACT

Weight gain and survival were examined in newly-hatched juvenile Australian crayfish, *Cherax quadricarinatus*, fed formulated crustacean feeds. Crayfish cultured using several Argent specialty feeds, including brine shrimp flakes, freeze-dried krill, powdered spirulina, and hatchfry encapsulon, exhibited high mortality (>90%) and little or no weight gain. After 10 weeks of culture, crayfish fed AB crayfish feed (AB) exhibited the highest weight gain with nearly 100% survival. Weight gain of crayfish fed other formulated feeds, such as Zeigler post-larval feed (ZPL), Zeigler shrimp grower (ZSG), Burris Mill crayfish feed (BM), Rangen shrimp grower (RSG), and a formulated crustacean feed (CRUS) were significantly lower. Survival of crayfish cultured using these feeds was also significantly lower, ranging from 40% (CRUS) to 72% (BM). Mortalities associated with these feeds occurred both during the intermolt period and during the molt. Recovered biomass was approximately half of that observed for crayfish cultured using AB feed, further indicating the inadequacy of these formulated feeds for use in crayfish cultures. These data suggest that many commercially available feeds do not provide the nutritional requirements for juvenile crayfish.

INTRODUCTION

Australian freshwater crayfish of the genus *Cherax* have received considerable interest among aquaculturists in the United States. The northern Australian species, *C. quadricarinatus*, has been studied extensively as a potential candidate for commercial culture (for review, see Semple et al., 1995). Several attractive characteristics of *C. quadricarinatus* include: a potentially larger size than native US species, a relatively non-aggressive behavior, and an ability to spawn multiple times annually. Furthermore, because this crayfish species naturally inhabits sub-tropical to tropical regions in Australia, the culture of *C. quadricarinatus* during the summer months in the southern latitudes of the US appears promising. The use of formulated feeds, rather than exclusive reliance on natural food sources, has been suggested as an effective method for sustaining and

enhancing crayfish growth within high density populations (D'Abramo and Robinson, 1989). Indeed, trial studies of many pond cultured crayfish species, including the Australian species *Cherax destructor*, have demonstrated that production can be increased when formulated feeds are used (Mills and McCloud, 1983). In those latitudes where overwintering is necessary, and naturally occurring foods are not readily available, formulated, nutritionally complete feeds will be necessary to maintain *C. quadricarinatus* broodstock in indoor facilities. These feeds must also satisfy the nutritional requirements of newly-hatched juvenile crayfish. Knowledge of the dietary requirements is, therefore, essential to insure successful commercial culture of *C. quadricarinatus*. To date, very little is known about the dietary requirements of *C. quadricarinatus* juveniles or adults.

The intensive culture of crustaceans, such as shrimp and prawns, relies on the use of specifically formulated commercial feeds. Use of commercial feeds for the culture of crayfish is, however, limited, particularly because few manufacturers market commercial feeds formulated specifically for crayfish. Since many crustaceans exhibit similar nutritional requirements, it is possible that some of these commercially available feeds could be used for the culture of crayfish, including *C. quadricarinatus*. In this study, we have examined weight gain and survival of newly-hatched juvenile *Cherax quadricarinatus* fed different commercially available crayfish and crustacean feeds.

MATERIALS AND METHODS

Adult broodstock crayfish were maintained in 2.5 m x 0.6 m raceways in the aquaculture facility located on campus at the University of Alabama at Birmingham. These stocks were originally obtained from Dr. David Rouse (Department of Fisheries and Allied Aquacultures, Auburn University). Each raceway in this facility was associated with its own recirculating biofiltration system to ensure water quality and the health of broodstock crayfish. All broodstock, spawned females, and juveniles used for feed experiments were maintained at 27 ± 1°C with daylight fluorescent lighting on a 12:12 photoperiod (Morrissy et al., 1990).

Female crayfish characteristically "tuck" their tails after spawning and extruding eggs (Masser et al., 1990). All raceways were examined daily for females exhibiting this behavior. When a female was suspected of carrying eggs, she was gently netted and examined for eggs. Once discovered, females carrying eggs were removed from the raceways and held individually in 6 *l* aerated, static water aquaria to minimize stress during the embryonic development period (~ 30 days). For each diet treatment, 30 newly-hatched juvenile crayfish (10 ± 1 mg fresh weight) from 3 females (10 siblings/female) were collected and placed individually in 10 cm diameter (bottom surface area ~ 80 cm^2) polystyrene bowls containing 200 ml of aerated tapwater. Water was replaced every other day with aerated tapwater to ensure that parameters such as alkalinity, hardness, pH, etc. remained within optimal levels (Masser et al., 1990). Ammonia and nitrites were monitored daily using test kits supplied by Fritz Aquaculture Inc. Previous studies have shown that, using the aforementioned schedule of water changes, levels of ammonia and nitrites remained undetectable in these individual containers.

Weight gain and survival were examined for 10 weeks using several commercial and formulated crustacean feeds (Table 1). The dietary composition of these feeds is proprietary information of the individual feed corporations and the UAB Research Foundation and, hence, is not included for comparison. To avoid handling effects associated with differential physical properties, all feeds were pulverized with mortar and pestle, and then mixed by equal weight in a 2% agar solution to make soft pellets (> 5 mm^3). The soft pellets containing the different feeds were then fed to the individual crayfish. Preliminary studies indicated that the juvenile crayfish could easily consume feeds proffered in this manner. The crayfish were fed daily *ad libitum*. Any food remaining from earlier feedings was removed and replaced with fresh food.

Newly-hatched juveniles were monitored twice daily for molts and mortalities. At the end of the 10 week growth period, individuals were also measured for wet weight (mg). Final mean weight weights are represented as mean ± standard error of the mean

Table 1. Formulated feeds used to culture newly-hatched juvenile Australian crayfish.

AB crayfish feed
Argent Brine Shrimp Flakes
Argent Freeze-Dried Krill
Argent Hatchfry Encapsulon
Burris Mills Crayfish Feed
[a]HFX CRD 84
Rangen Shrimp Grower
Rangen Production Grower
Rangen Post-Larval Grower
Zeigler Shrimp Grower
Zeigler Post-Larval Feed

[a] provided by Dr. Louis D'Abramo, Dept. of Wildlife and Fisheries, Miss. State Univ.

(error bars in figures). Mean final wet weights were analyzed using one-way ANOVA and multiple comparisons among the feed treatments were made using Tukey's test (Daniel, 1987). Survival data were arcsine transformed and analyzed using the Chi-square test. Alpha (α) for statistical analysis was set at 0.05.

RESULTS

Weight gain and survival of newly-hatched juvenile crayfish cultured using Argent's specialty feeds, including brine shrimp flakes, freeze-dried krill, powdered spirulina, and hatchfry encapsulon, as a complete diet were minimal. Crayfish fed any of these feeds exhibited high mortality (70-90%) within 4-5 weeks. Mortality of these crayfish occurred primarily during the intermolt period and did not appear related to an inability to successfully molt. Little or no weight gain during this period was observed for crayfish cultured using these feeds (the largest of individuals was approximately 13 mg after 5 weeks of culture). Greatly lengthened molt intervals suggested further that these feeds were highly deficient in the specific nutritional components necessary to maintain maximal weight gain and survival of the juvenile crayfish. Abnormal carapace pigmentation, a characteristic commonly observed in nutritionally deprived crustacean cultures (New and Singholka, 1985) was also observed in crayfish fed the Argent specialty feeds.

Final mean wet weights (Fig. 1a) and survival (Fig.1b) of newly-hatched crayfish cultured using several other commercial feeds were much improved. At the end of 10 weeks, crayfish fed AB (UAB Research Foundation formulation) exhibited the highest weight gain and appeared the healthiest, as indicated by their carapace pigmentation (dark brown). Crayfish fed any of the remaining feeds demonstrated significantly reduced weight gain and exhibited abnormal carapace pigmentation (pale or no coloration). In summary, AB fed crayfish averaged 568.5 ± 34.8 mg with 97% survival, ZSG (Zeigler shrimp-grower) fed crayfish averaged 323.0 ± 37.1 mg with 66% survival, RSG (Rangen shrimp-grower; 50/15) fed crayfish averaged 295.6 ± 21.8 mg with 73% survival, BM

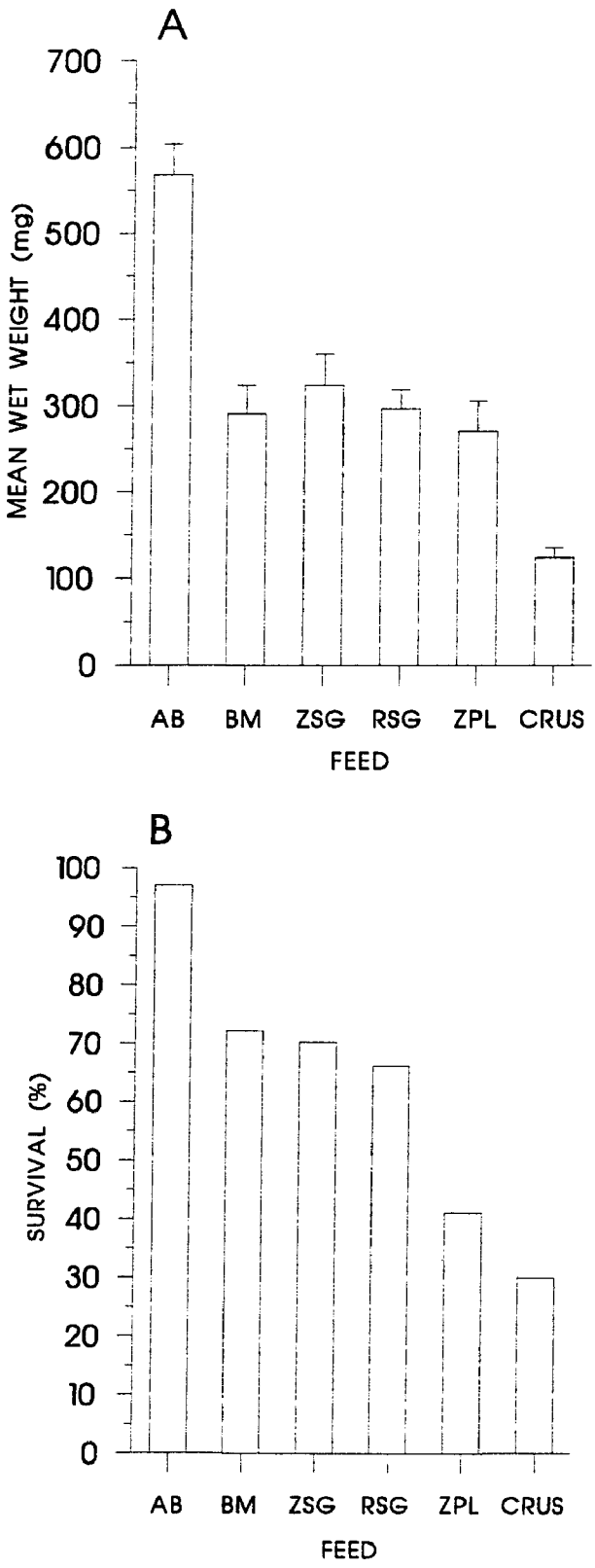

Figure 1. Mean wet weight ± se (A) and survival (B) of juvenile crayfish fed different feeds.

(Burris Mills crayfish feed) fed crayfish averaged 290.0 ± 33.3 mg with 73% survival, ZPL (Zeigler post-larval feed) fed crayfish averaged 270.0 ± 34.4 mg with 40% survival, and CRUS (HFX CRD 84) fed crayfish averaged 123.7 ± 11.8 mg with 30% survival.

Statistically, higher weight gain and survival of AB fed crayfish resulted in a higher recovered biomass (Fig. 2). No significant differences in weight gain were observed among crayfish fed the ZSG, RSG, BM, and ZPL feeds. Weight gain of CRUS-fed crayfish was significantly less than crayfish fed all other feeds. Similarly, no significant differences in survival were observed among crayfish fed the ZSG, RSG, and BM feeds. Survival of crayfish fed ZPL and CRUS, however, was significantly less than crayfish fed all other feeds.

DISCUSSION

Mortality of the crayfish fed any of the feeds could not be attributed to any particular circumstance. The demise of many crayfish occurred during the molt. Several crayfish, however, also died during the intermolt period or just shortly after a molt. Nutritional deficiencies can lead to the inability of a molting individual to completely extricate itself from the old exoskeleton. In many cases, the individual will suffocate as a result of the incomplete ecdyses. This condition is often referred to as a "molting death syndrome". Many investigators have observed this syndrome in cultured crustaceans when using feeds lacking specific dietary lipids (Conklin, et al., 1980; D'Abramo et al., 1985). Since the specific dietary components of the different feeds were not known, we cannot speculate on the influence of dietary lipids and the molting success of the juvenile crayfish in this study. Nevertheless, the lack of specific dietary components likely resulted in the differential survival observed.

Comparisons of weight gain and survival of newly-hatched juvenile *Cherax quadricarinatus* cultured indoors using different feeds have not been reported. Weight gain and survival have been reported for individually cultured juvenile *C. destructor* using a formulated ration proffered in different quantities (Geddes et al., 1988). After 90 days

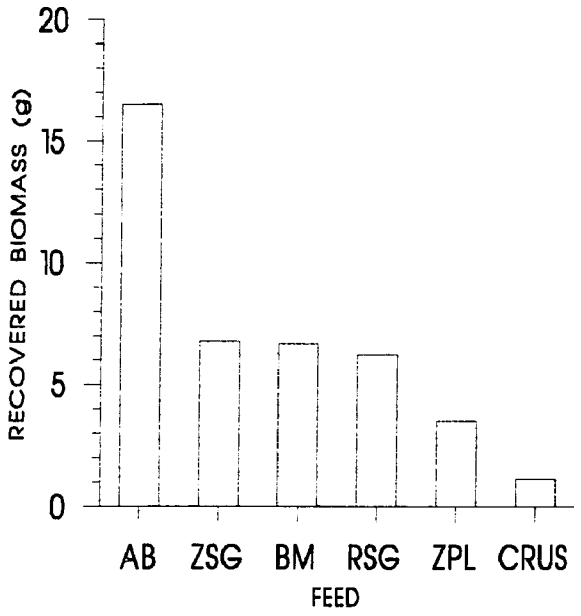

Figure 2. Total recovered biomass (g) of juvenile crayfish fed different feeds.

of culture when fed 10% of their body weight, individuals attained a mean maximal weight of 7.4 g with nearly 100% survival. Juveniles used in these experiments were, however, initially larger (~100 mg) than the individuals used in this study (~10 mg). When cultured communally and fed 10% of their body weight, *C. destructor* attained a mean maximal weight of 3.6 g with significantly reduced survival. Competition for available food and shelter resources were attributed to the reduced weight gain and survival in the communal cultures (Geddes et al., 1988). The formulation of feeds that meet the complete nutritional requirements of crayfish may, however, reduce competition among conspecifics and aid in maintaining near maximal growth of individuals in communal cultures.

Instantaneous growth rates (IGR) and survival of *Cherax quadricarinatus* in this study (AB diet) were compared with IGR and survival of *C. quadricarinatus* observed in other studies (Table 2). In all of these studies, the diets used were specifically formulated for crayfish. IGR and survival data obtained from a preliminary study culturing *Procambarus clarkii*, a commercially important crayfish species in the US, using AB feed was included in this comparison. Although comparisons among such studies are difficult, particularly considering that methods of experimental design and data analysis are not standardized practices among researchers, such comparisons are necessary to assess the effectiveness of newly-formulated feeds. Although IGR values in this comparison were variable, weight gain of the crayfish was substantial and survival was greater than 95%. It is apparent from these studies that the culture of juvenile crayfish (*C. quadricarinatus* and *P. clarkii*) can be successful when using formulated feeds.

Of all the feeds evaluated, juvenile crayfish fed AB exhibited the highest weight gain and survival. Although, at present, this feed is not commercially available, the dietary constituents used to make this feed are readily available and are not subject to large changes in nutrient composition. Thus, if marketed, AB could be used as a complete diet for the culture of crayfish. The other feeds used in this study are commercially available; however, sub-optimal weight gain and survival were observed in juveniles fed these diets.

Table 2. Instantaneous growth rates (IGR) and survival of juvenile crayfish fed formulated diets.

Animal	Feed	[a]IGR (%/day)	Survival (%)	Source
P. clarkii	formulated diet	3.7	~95	Reigh et al., 1993
P. clarkii	formulated diet	6.4	~96	Lochmann et al., 1992
P. clarkii	AB feed	8.2	100	Meade and Watts, unpub. data
C. quadricarinatus	AB feed	5.8	~95	this study
C. quadricarinatus	formulated diet	2.6	NR	Jones, 1988
C. quadricarinatus	formulated diet	2.4	NR	Austin, 1995

[a]$IGR = 100 \times [\ln(W_t/W_o)]/t$
NR = not reported

Culturists should be aware that such feeds do not provide for the complete nutritional needs of crayfish. To account for the possible nutritional deficiencies of commercial feeds, many culturists suggest using other food sources, such as fish, squid, alfalfa pellets, or other natural animal or plant materials, as supplemental feeds. Since the nutritional requirements of crayfish are not known, such feeding practices can be performed only at the discretion of the individual culturist. Culturists should also be aware that such supplemental feeding practices can result in water quality problems. Overall, it is apparent that further evaluation of the nutritional requirements and the consequent formulation of feeds specifically for crayfish are needed before semi-intensive and intensive production can be improved.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Louis D'Abramo (Miss. State Univ.) for providing the crustacean reference diet and Dr. David Rouse (Auburn Univ.) for providing broodstock crayfish. The authors also wish to thank Argent Chemical Laboratories, Burris Mill and Feed Inc., Massey Milling Co., Rangen Inc., Zeigler Bros. Inc., and the UAB Research Foundation for their generous donations of feeds and the formulations of feeds used in this study. This research was funded in part by the Alabama Universities/TVA Research Consortium (AUTRC).

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PATTERNS OF GROWTH IN JUVENILE AUSTRALIAN CRAYFISH, *Cherax quadricarinatus*, FED FORMULATED CRUSTACEAN FEEDS

Authors: Mark E. Meade
Stephen A. Watts

Submitted to: Journal of Crustacean Biology

ABSTRACT

Patterns of growth and survival were examined in newly-hatched juvenile Australian crayfish, *Cherax quadricarinatus*, fed three formulated crustacean feeds. After 10 weeks of culture, crayfish fed AB crayfish feed (AB) exhibited a significantly higher weight gain and survival than crayfish fed Zeigler post-larval feed (ZPL) or a formulated crustacean feed (CRUS). Crayfish fed AB exhibited the shortest molt intervals (time between successive molts) and the greatest molt increments (weight gains per molt). Crayfish fed ZPL exhibited molt intervals similar in duration to crayfish fed AB, but had significantly reduced molt increments. Crayfish fed CRUS exhibited both longer molt intervals and reduced molt increments compared to crayfish fed AB. Average instantaneous growth rates (IGR) were highest in the AB treatment for the duration of the 10 week culture period. After 10 weeks of culture, the surviving individuals in the ZPL and CRUS treatments were fed AB. After another 6 weeks of culture, these crayfish exhibited improved weight gain and survival. Molt intervals for the ZPL/AB treatment were similar in duration to those of crayfish in the AB/AB treatment. Molt increments for the ZPL/AB treatment were, however, greater than those observed for similar size crayfish in the AB treatment. Molt intervals for the CRUS/AB treatment were shorter in duration and molt increments were greater than those observed for similar size crayfish in the AB treatment. IGR values increased substantially for the CRUS/AB and ZPL/AB treatments during this 6 weeks of culture, indicating compensatory growth. These data demonstrate that diet can effect the weight gain and survival of juvenile *C. quadricarinatus* by influencing both molt intervals and/or molt increments.

INTRODUCTION

Growth of freshwater crayfish, and many other decapod crustaceans, is a complex phenomenon, influenced by many biological and physical environmental factors. Several investigators have examined the effects of factors, such as salinity, temperature, and nutrition, on the growth and survival of crayfish and other crustaceans (for review, see

Hartnoll, 1982). Few investigators, however, have examined components of crayfish growth. As crayfish exhibit discontinuous growth (Hartnoll, 1982), the mechanisms for increased growth of any one individual can occur by 1) shortened molt intervals (the time between molts) and/or 2) increased molt increments (weight gain at the individual molts).

Because of their large size at maturity, the Australian freshwater crayfish, *Cherax quadricarinatus*, has received a great deal of attention as a potential culture species throughout the world (Semple et al., 1995). Growth of juvenile *C. quadricarinatus* has been examined extensively in laboratory conditions (Jones, 1988; 1989; Medley et al., 1994). When cultured using different formulated crustacean feeds, newly-hatched juvenile *C. quadricarinatus* demonstrate significant differences in final mean wet weights and survival (Meade and Watts, in review), suggesting that these feeds differ in their nutritional quality. The mechanisms (changes in molt intervals and/or molt increments) which result in differential growth of juveniles fed different feeds have not been determined. Clearly, information concerning dietary influences on molt intervals and/or molt increments will increase our knowledge on the effects of feeds in regulating growth processes. In this study, we describe these components of early growth in newly-hatched juvenile crayfish, *C. quadricarinatus*, cultured using a feed previously determined to maximize weight gain. We also describe the components of growth in juveniles fed two feeds that result in different levels of reduced weight gain. Finally, we describe a compensatory strategy of growth in juvenile crayfish which have been nutritionally deprived.

MATERIALS AND METHODS

Broodstock *Cherax quadricarinatus* were maintained in static water, biofiltered raceways located in the aquaculture facility at the University of Alabama at Birmingham. Spawned females were maintained individually in 6 *l* aerated, static water aquaria to minimize stress during embryonic development. Each experimental treatment consisted of 30 newly-hatched juvenile crayfish (10 ± 1 mg fresh weight) randomly selected from 3

broodstock females. All juveniles in each treatment were held individually in 10 cm diameter (bottom surface area ~ 80 cm^2) polystyrene bowls containing 200 ml of aerated tapwater. Water was changed every other day to ensure that water quality remained optimal. Ammonia and nitrites were monitored daily using colorimetric test kits (Fritz Aquaculture, Inc.). For the duration of the experiments, ammonia and nitrites were never detected in the individual containers. All juvenile crayfish were cultured at 27 ± 1°C with daylight fluorescent lighting on a 12:12 photoperiod (Morrissy, 1990).

Three formulated feeds were proffered to the crayfish: AB crayfish feed (AB), a crayfish feed formulation provided by the UAB Research Foundation; HFX CRD 84 (CRUS), a formulated crustacean feed provided by Dr. Louis D'Abramo, Dept. of Fisheries and Wildlife, Miss. State Univ.; and Zeigler post-larval feed (ZPL), provided by Zeigler Bros. Inc., Gardners, PA. Proximate biochemical composition of the feeds is given in Table 1. All feeds were pulverized and mixed in 2% agar to avoid potential problems associated with handling resulting from the different physical properties of the feeds. The agar/feed mix was cut into soft pellets (> 5 mm^3) which were fed to the individual crayfish. The crayfish were fed *ad libitum* daily. Food remaining in the containers on subsequent feeding days was removed and replaced with fresh food.

Weight gain and survival of the juvenile crayfish were monitored for 10 weeks. Twice daily, the crayfish were monitored for molts and mortalities. Individual crayfish were measured weekly for wet weight (mg) and total length (mm) until the end of the 10 weeks of culture. Data are presented as mean ± standard error of the mean (error bars in figures). Mean wet weights at the end of 10 weeks were analyzed using one-way ANOVA and multiple comparisons among the diet treatments were made using the Tukey's test (Daniel, 1987). Survival data were arcsine transformed and analyzed using the Chi-square test (Zar, 1974). Alpha (α) for statistical analysis was 0.05.

Following statistical analyses of weight gain and survival data, the remaining survivors in each treatment (n = 29, 12, and 9, respectively, for the AB, ZPL, and CRUS

Table 1. Proximate biochemical composition of feeds used to culture juvenile crayfish, Cherax quadricarinatus.

Component	AB crayfish feed[a]	Crustacean diet[b]	Zeigler shrimp post-larval feed[c]
Protein	30%	36%	41%
Fat	10%	5%	10%
Carbohydrate	10%	13%	3%
Ash	3%	6%	11%
Moisture	9%	13%	10%

[a]Protein determined by the methods of Bradford (1976), lipid by the methods of Freeman et al. (1957), carbohydrate by the methods of Dubois et al. (1950), ash content determined by drying feed at 400°C for 4hr, and moisture determined by lyophilizing feed for 24hr.
[b]HFX CRD 84, provided by Dr. L. R. D'Abramo, Department of Wildlife and Fisheries, Mississippi State University, Mississippi.
[c]product no. 34-6900, provided by Zeigler Bros. Inc., Gardners, Pennsylvania.

diet treatments) were fed that feed (AB) which promoted the highest weight gain and survival during the first 10 weeks. We hypothesized that the crayfish fed sub-optimal feeds previously may improve their weight gain and survival when fed a feed shown to elicit high weight gain and survival. These feeds were designated as AB/AB (those fed AB for the entire 16 week growth period), ZPL/AB (those fed ZPL for 10 weeks, then converted to AB), and CRUS/AB (those fed CRUS for 10 weeks, then converted to AB). Weight gain and survival of the crayfish were monitored for another six weeks. The crayfish were fed and monitored using protocols described previously. At the end of the 6 week culture period, mean wet weights and survival were again analyzed for statistical comparisons among the three treatments.

To assess the components of growth observed among the three feed treatments, molt intervals (the time between successive molts) and molt increments (the weight gain per molt) were analyzed using regression models developed by Mauchline (1977). For many of the data sets analyzed, as the crayfish grew, the variances observed for molt intervals and molt increments increased. Logarithmic and semi-logarithmic transformations of the data did not aid in reducing the variance or strengthen the correlation of the regressions. Thus, all of the molt interval and molt increment data are presented using simple linear regression models. The relationship used to describe molt intervals was the comparison of molt intervals to premolt carapace lengths. The relationship used to describe molt increments was the comparison of weight increments to premolt weights. Slopes of the regression were compared using an F test, and when found significant, by multiple comparisons using Tukey's test. Instantaneous growth rates (IGR) were also calculated for each diet treatment at intervals of 2 weeks using equation 1. In this equation, W_t is the weight at any time t and W_0 is the weight at time 0. IGR data were analyzed statistically by one-way ANOVA. Alpha (α) for all statistical analyses was 0.5 (Daniel, 1987).

$$IGR = 100 \times [\ln(W_t/W_0)]/t \qquad (1)$$

RESULTS

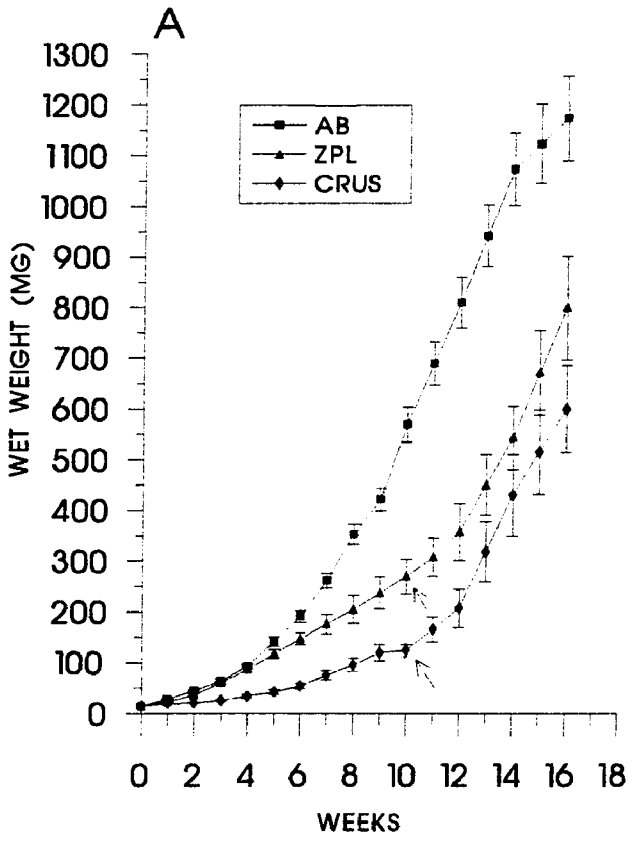
Morphometry and Growth Components - Weeks 1-10

Mean wet weights of hatchling *Cherax quadricarinatus* fed AB, ZPL, or CRUS were significantly different after 10 weeks of culture (Fig. 1a). Crayfish fed AB were the largest, averaging 569 ± 35 mg wet weight. Crayfish fed ZPL and CRUS were 53 and 78% smaller, averaging 270 ± 34 mg and 124 ± 12 mg wet weight, respectively. Survival of crayfish in the different diet treatments was 97, 40 and 30% for the AB, ZPL, and CRUS groups, respectively (Fig. 1b).

Regression analyses indicated that the correlation between molt intervals and premolt carapace lengths was relatively low and only the calculated regressions for the AB and ZPL treatments were statistically significant for the first 10 weeks of culture (Fig. 2 a, b, and c; Table 2ii). Although crayfish in the CRUS treatment exhibited the highest variance, all of the regressions could be used to estimate molt intervals for a particular size class of crayfish. For example, molt intervals of 7 to 8 days were observed for 12 mm size class individuals in the AB and ZPL treatments, whereas, molt intervals of approximately 12 days (~ 30% longer in duration) were observed for 12 mm size class individuals in the CRUS treatment.

Regression analyses indicated that the correlation between molt increments and premolt weights was significant for all three treatments for the first 10 weeks of culture (Fig. 3 a, b, c; Table 2i). The slope of the regression for the ZPL treatment was significantly lower than the slopes of the regressions for the AB and CRUS treatments. Although the slopes of the calculated regressions for the AB and CRUS treatments were not significantly different from each other, the regressions provided an estimate of molt increments, suggesting that the different feeds did influence the weight gained at each molt. For example, 100 mg individuals in the AB treatment would gain 50 to 70% (mean 59.23) of their premolt weight at each molt, individuals in the ZPL treatment would gain 30 to 50% (mean 44.86) of their premolt weight at each molt, and individuals in the

Figure 1. Mean wet weights ± se (A) and survival (B) of juvenile Australian crayfish fed AB crayfish feed (AB), Zeigler post-larval feed (ZPL), and a formulated crustacean feed (CRUS). Each treatment consisted of 30 individuals at the beginning of the experiments. Arrows indicate when all treatments were converted to AB.


A
AB
ZPL
CRUS
WET WEIGHT (MG)
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
0 2 4 6 8 10 12 14 16 18
WEEKS

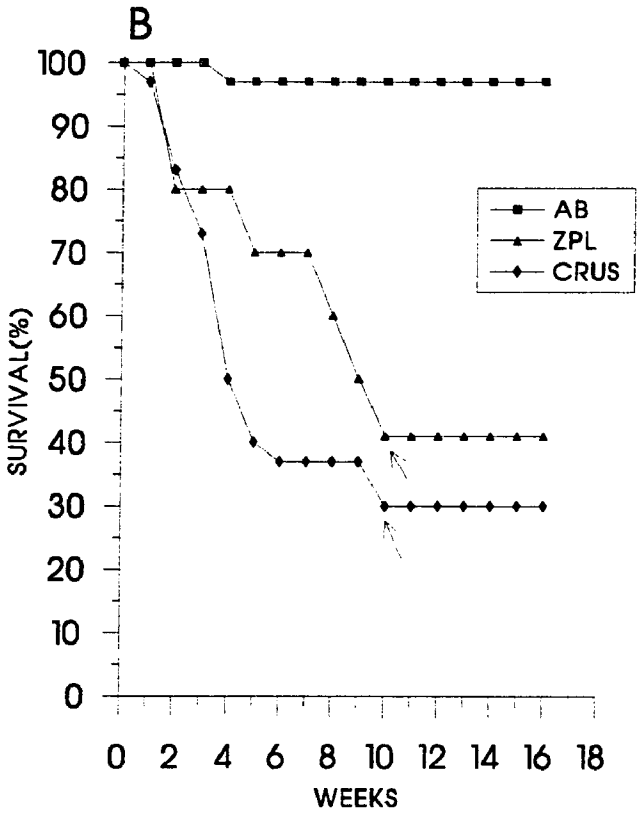

B
AB
ZPL
CRUS
SURVIVAL(%)
100
90
80
70
60
50
40
30
20
10
0
0 2 4 6 8 10 12 14 16 18
WEEKS

Figure 2. Relationship between molt intervals and premolt carapace lengths during the first 10 weeks of culture for crayfish in the AB (A), ZPL (B), and CRUS (C) treatments, and weeks 10 to 16 of culture for crayfish in the AB/AB (D), ZPL/AB (E) and CRUS/AB (F) treatments.

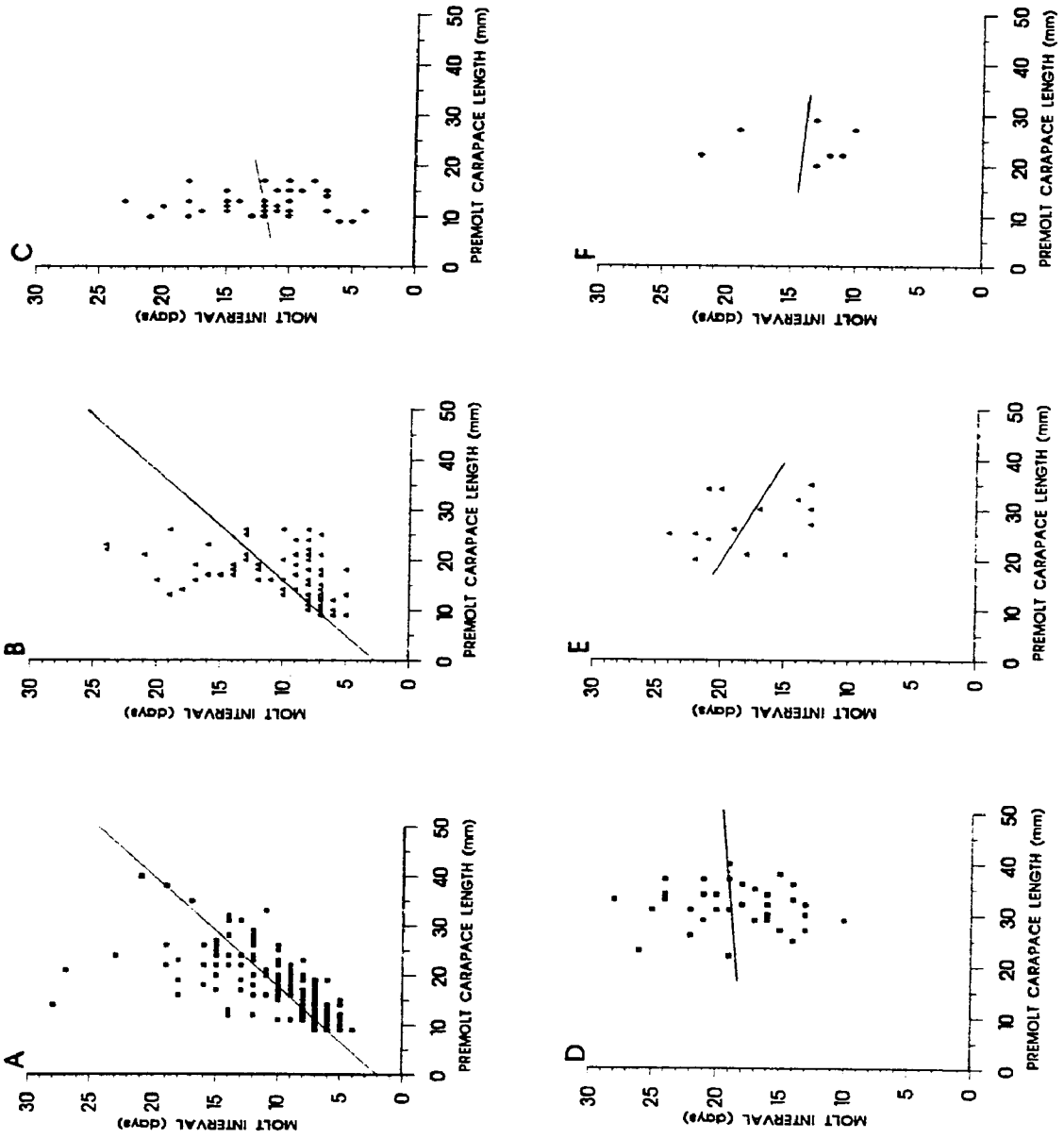

A
B
C
D
E
F
MOLT INTERVAL (days)
PREMOLT CARAPACE LENGTH (mm)
0
5
10
15
20
25
30
40
50

Figure 3. Relationship between molt increments and premolt weights during the first 10 weeks of culture for crayfish in the AB (A), ZPL (B), and CRUS (C) treatments, and weeks 10 to 16 of culture for crayfish in the AB/AB (D), ZPL/AB (E) and CRUS/AB (F) treatments.

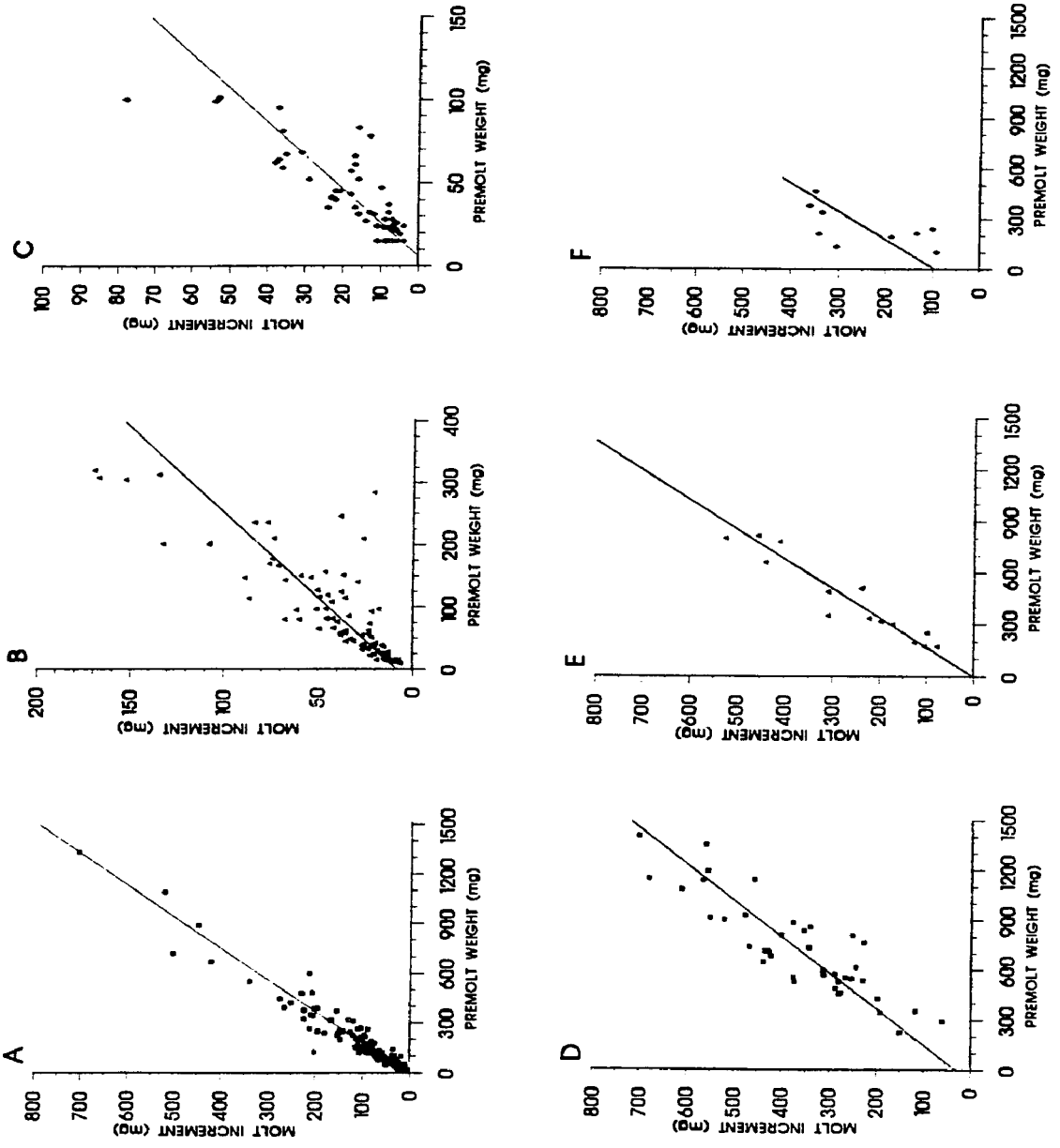

A
B
C
D
E
F
MOLT INCREMENT (mg)
PREMOLT WEIGHT (mg)

Table 2. Relationship between molt increment and intermolt period among juvenile crayfish fed different diets.

Regression	AB (n=29)	ZPL (n=12)	CRUS (n=9)
(i) weight increment (Y) premolt weight (X) (1-10 weeks)	Y=6.81 + 0.52X s.e. slope 0.010 r^2 0.939 t 54.45	Y=8.50 + 0.36X s.e. slope 0.026 r^2 0.689 t 13.82	Y=-2.72 + 0.50X s.e. slope 0.045 r^2 0.698 t 10.86
(ii) intermolt period (Y) premolt length (X) (1-10 weeks)	Y=2.07 + 0.45X s.e. slope 0.037 r^2 0.448 t 12.09	Y=2.71 + 0.46X s.e. slope 0.103 r^2 0.201 t 4.48	Y=11.02 + 0.08X s.e. slope 0.298 r^2 0.002 t 0.281*
(iii) weight increment (Y) premolt weight (X) (10-16 weeks)	Y=31.67 + 0.46X s.e. slope 0.041 r^2 0.768 t 11.23	Y=2.09 + 0.58X s.e. slope 0.057 r^2 0.891 t 10.29	Y=94.4 + 0.59X s.e. slope 0.288 r^2 0.374 t 2.05*
(iv) intermolt period (Y) premolt length (X) (10-16 weeks)	Y=17.60 + 0.04X s.e. slope 0.183 r^2 0.001 t 0.209*	Y=24.67 - 0.24X s.e. slope 0.205 r^2 0.105 t -1.19*	Y=15.09 - 0.05X s.e. slope 0.395 r^2 0.002 t -0.114*

* only t value for slope not significant at an α of 0.05.

CRUS treatment would gain 35 to 50 % (mean 46.84) of their premolt weight at each molt.

Morphometry and Growth Components - Weeks 10-16

Mean wet weights of the crayfish fed AB, ZPL, or CRUS for the first 10 weeks and then AB for the next 6 weeks were significantly different at the end of 16 weeks of culture (Fig. 1a). Crayfish in the AB/AB treatment continued to increase in size, averaging 1172 ± 84 mg wet weight, despite being held in containers that may have influenced their potential maximal weight gain. Although the final size of crayfish in the ZPL/AB and CRUS/AB treatments was 32 and 49% smaller than those fed AB/AB (averaging 798 ± 103 mg and 599 ± 86 mg wet weight, respectively), these groups continued to increase in size during this 6 weeks of culture (Fig. 1a). No mortalities were observed during this period (Fig. 1b).

Regression analyses for weeks 10 to 16 of culture indicated that the correlation between molt intervals and premolt lengths was relatively low and that none of the calculated regressions was statistically significant for the AB/AB, ZPL/AB, or CRUS/AB treatments, respectively (Fig. 2 d, e, f ; Table 2iv). This was attributed to the small number of individual observations during this 6 weeks of the experiment. These regressions, however, could be used to estimate molt intervals for a particular size class of crayfish. For example, molt intervals of approximately 18 days were observed for 25 mm size class individuals in the AB/AB and ZPL/AB treatments, whereas, molt intervals of approximately 14 days (~ 22% shorter in duration) were observed for 25 mm size class individuals in the CRUS/AB treatment.

Regression analyses for weeks 10 to 16 of culture indicated that the correlation between molt increments and premolt weights was significant for the AB/AB and ZPL/AB treatments, but not for the CRUS/AB treatment (Fig. 3 d, e, f; Table 2iii). The non-significant t value for the relationship in the CRUS/AB treatment could again be attributed to the small number of observations during this period. Nevertheless, the regression

provided an estimate of molt increments, suggesting that the feed did influence the weight gained at each molt. The slope of the regression for the ZPL/AB treatment was significantly greater than the slope of the regression for the AB/AB treatment. Indeed, individuals in the ZPL/AB and CRUS/AB treatments had higher percentage molt increments during this period than previous individuals in the AB treatment of similar size. For example, a comparison of crayfish in the 500 mg size class demonstrated that individuals in the AB treatment gained an average of 52% of their premolt weight at each molt, whereas, 500 mg size class individuals in the ZPL/AB treatment gained an average of 58% of their premolt weight at each molt, and crayfish in the CRUS/AB treatment gained an average of 97% of their premolt weight at each molt.

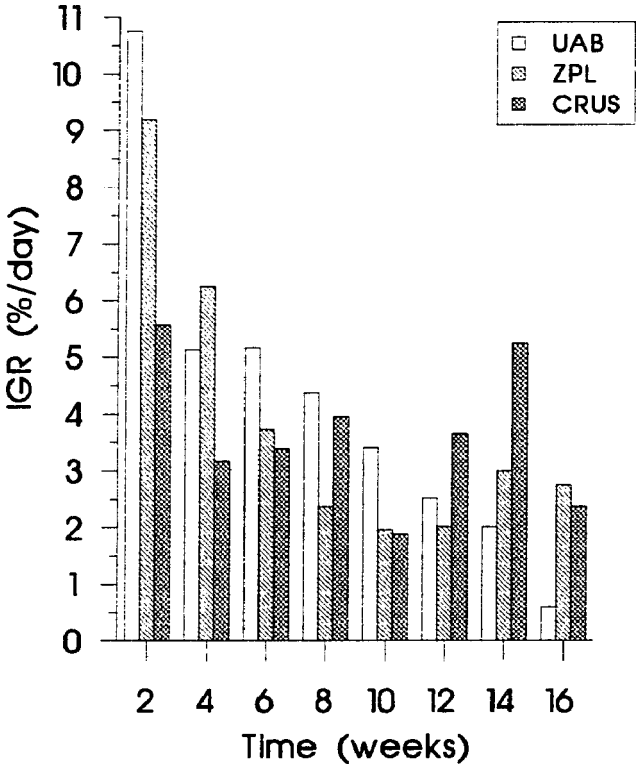
Instantaneous growth rates (IGR)

For all treatments, the highest IGR was observed during the first 2 weeks of culture (Fig. 4). Following the first week, IGR decreased for all treatments until week 6 of culture. At this time, IGR for the CRUS treatment remained relatively constant until decreasing at week 10. IGR for the AB and ZPL treatments continued to decline during this period. The highest average IGR during the first 10 weeks of culture was observed for the AB treatment. After week 10, IGR for the AB/AB diet treatment continued to decline. Conversely, IGR for the ZPL/AB and CRUS/AB treatments began increasing at week 10 and remained relatively high for the duration of the experiments. The highest average IGR for the last six weeks of culture was observed for the CRUS treatment.

DISCUSSION

Growth of crustaceans is discontinuous, and can be separated into two components, molt interval and molt increment. These growth processes are essentially discrete, often exhibiting very different responses to physiological and/or environmental fluctuations (for review see Hartnoll, 1982). The development of growth models for a particular species thus requires that these two processes be examined separately.

Figure 4. Instantaneous growth rates (IGR) for the population of juvenile Australian crayfish fed AB crayfish feed (AB), Zeigler post-larval feed (ZPL), or a formulated crustacean feed (CRUS) during a 16 week culture period. All treatments were fed AB after week 10 of culture.


UAB
ZPL
CRUS
IGR (%/day)
0
1
2
3
4
5
6
7
8
9
10
11
2
4
6
8
10
12
14
16
Time (weeks)

Mean wet weights of juvenile *Cherax quadricarinatus* fed AB after the first 10 weeks of culture in this study were significantly greater than those of juveniles fed ZPL or CRUS. Although growth components have not been previously reported for this species, molt intervals and molt increments for juveniles fed AB in this study were similar to those reported for juvenile *Cherax destructor* when fed and cultured optimally (Geddes et al., 1988).

Mean wet weights of juveniles fed ZPL during the first 10 weeks of culture were approximately 50% less than mean wet weights of juveniles fed AB. Regression analyses indicated that crayfish in this treatment were smaller because of decreased molt increments, despite maintaining molt intervals similar to those observed for crayfish receiving the AB treatment. These data suggest that ZPL provided those nutritional components necessary to maintain molting frequency, but not to maintain maximal molt increments. Mean wet weights of juveniles fed CRUS during the first 10 weeks of culture were approximately 80% less than mean wet weights of individuals fed AB. Regression analyses indicated that crayfish in the CRUS treatment were smaller because of both lengthened molt intervals and decreased molt increments. These data suggest that CRUS was deficient in those nutritional components necessary to maintain molt intervals and molt increments.

In decapod crustaceans, several investigators have demonstrated that molt intervals and/or molt increments can be altered with changes in either the quantity or quality of available food (Hartnoll, 1982). There is much debate, however, as to whether the depression in growth occurs predominately by lengthening molt intervals or decreasing molt increments. The data presented in this study indicate that one or both of these changes can occur, resulting in decreased growth of juvenile crayfish. We suggest that ZPL may be deficient only in those nutritional components required to maximize molt increments, whereas, CRUS may be deficient in those nutritional components required to maintain both molting frequencies and molt increments. The proximate biochemical

composition of the feeds used in this study demonstrated only minor differences in the total protein, carbohydrate, and lipid composition, and no apparent correlation with weight gain could be determined. Furthermore, since the source or type of the individual components, such as whether protein in the feeds is of plant or animal origin, is considered proprietary to the individual feed manufacturers, those dietary components which resulted in differential molting frequencies and molt increments in juvenile crayfish during the first 10 weeks of this study could not be determined.

Juvenile crayfish in the ZPL/AB and CRUS/AB treatments demonstrated increased weight gain during the last 6 weeks of culture. In comparison to the AB/AB treatment, molt intervals for the ZPL/AB treatment were not different during this period; however, molt increments for the ZPL/AB treatment increased by approximately 13%. Corroboratively, instantaneous growth rates (IGR) for the ZPL/AB diet treatment also increased during this period. In contrast, molt intervals for the CRUS/AB treatment were substantially shorter than those of the AB/AB and ZPL/AB treatments during this period. Moreover, molt increments for the CRUS/AB treatment were approximately 45% greater that those of the AB/AB treatment. The IGR for the CRUS/AB diet treatment also increased during this period. Overall, these data suggest that crayfish in the ZPL/AB and CRUS/AB treatments compensated for their submaximal weight gain observed during the first 10 weeks of the study. Although crayfish in the ZPL/AB and CRUS/AB treatments never attained sizes comparable to individuals in the AB/AB treatment, their rates of weight gain suggested that they would have attained sizes similar to crayfish in the AB/AB treatment in time. IGR, however, decreased for individuals in the AB/AB treatment during the last few weeks of growth. We suggest that the size of the containers likely affected these individuals, thus, reducing their potential weight gain. From the observations of crayfish in the ZPL/AB and CRUS/AB treatments, which exhibited poor growth during the first 10 weeks of culture, it appears that crayfish can compensate for depressed growth resulting from poor nutrition by increasing molting frequency and/or

molt increments when converted to nutritionally complete diets. Future analyses on the effects of specific nutrients on growth components in crayfish are needed to determine which nutritional components are required to maintain maximal molting frequencies and molt increments, thus allowing for maximal weight gain.

ACKNOWLEDGMENTS

We thank Dr. Louis D'Abramo, the UAB Research Foundation, and Zeigler Bros. Inc. for their generous donations of the feeds and feed formulations used in this study. We also thank Dr. Rob Angus for assistance with statistical analyses of data.

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HEAT AND OXYGEN FLUX AS A FUNCTION OF ENVIRONMENTAL p_{O_2} IN JUVENILE AUSTRALIAN CRAYFISH, *Cherax quadricarinatus*

Authors: Mark E. Meade
Jeannette E. Doeller
David W. Kraus
Stephen A. Watts

Published in: The Journal of Experimental Zoology 270(5):460-466 (1994)

ABSTRACT

Oxygen and heat flux of the juvenile Australian red claw crayfish, *Cherax quadricarinatus*, were measured in parallel respirometry and calorimetry systems at 28°C at varying levels of enviromental p_{O_2}. Juvenile crayfish maintained an average oxygen flux of 5.0 ± 0.4 (SEM) pmol O_2/(s*mg fresh weight) over the p_{O_2} range from 20.7 to 5 kPa. At approximately 4 kPa p_{O_2}, oxygen flux decreased, conforming to decreasing oxygen tensions. Juvenile crayfish maintained an average heat flux of -2.2 ± 0.2 μW/mg fresh weight when exposed to 100 and 50% air saturated conditions (18.1 to 20.7 and 7.8 to 10.4 kPa p_{O_2}, respectively). The ratio of the amount of heat dissipated per oxygen consumed, called the calorimetric/respirometric (CR) ratio, at 100 and 50% air saturated conditions averaged -440 kJ/mol O_2. When crayfish were exposed to 25% air saturated conditions (2.5 to 5.2 kPa p_{O_2}), heat flux decreased initially to -1.1 ± 0.4 μW/mg fresh weight. Within 4 hr, however, heat flux returned to normoxic values. When crayfish were exposed to anoxic conditions, heat flux decreased to zero. Most juvenile crayfish did not survive short term (1-2 hr) anoxic stress. These data suggest that juvenile *C. quadricarinatus* are excellent oxygen regulators over a wide range of environmental p_{O_2}. These data indicate also that juvenile *C. quadricarinatus* can compensate metabolically, possibly via aerobic and/or anaerobic adjustments, in order to maintain metabolic rate at critically low p_{O_2}. Lastly, this crayfish appears limited in its ability to utilize anaerobic pathways to maintain metabolic rate in anoxic environments.

INTRODUCTION

The diverse family of Parastacid decapods has been documented from South America, New Zealand, Madagascar, and Australia. This family comprises some of the largest populations of freshwater crayfish in the world, including over 100 species in at least 10 genera in Australia (for review, see Reik, 1969; 1972). *Cherax* is the most prevalent of the Australian genera, with species occupying a variety of habitats in southwestern, southeastern, eastern, and northern Australia. This genera also contains

some of the largest crayfish species found in the world, with some exceeding 2 kg fresh weight (Reik, 1969).

Cherax quadricarinatus, a species indigenous to northern Australia, is adapted to living in warm climates and is known to inhabit areas that experience drought during certain seasons (Reik, 1969). To survive drought conditions, this crayfish constructs deep burrows which end in large chambers harboring reserve water. Burrowing is also observed in this crayfish when escaping predation (Reik, 1969; 1972). As a consequence of burrowing deep into the sediments, crayfish can be exposed to reduced environmental oxygen tensions (McMahon, 1993; Reiber, 1993). The extent to which *Cherax quadricarinatus* can tolerate exposure to hypoxic or anoxic environments, including any metabolic adjustments that allow its survival, is not known.

Aquatic invertebrates are typically categorized according to their metabolic response when exposed to reducing environmental oxygen tensions (Mangum and Van Winkle, 1973; Herreid, 1980). Metabolic conformers are those organisms which vary their oxygen consumption rate directly with environmental oxygen partial pressures; whereas, metabolic regulators are those organisms which maintain their oxygen consumption rate independent of environmental oxygen partial pressures. In some instances, aquatic invertebrates will utilize anaerobic pathways for energy production when exposed to environmental hypoxia or anoxia. The ability to make such metabolic adjustments is quite variable from species to species (eg. Mangum and Van Winkle, 1973; Pamatmat, 1978; Herreid, 1980; Spotts, 1983; Morrissy et al., 1984; Doeller et al., 1990; Wang and Widdows, 1993). Other aquatic invertebrates do not exhibit the capacity for anaerobic metabolism and cannot maintain energy production when exposed to environmental hypoxia or anoxia. Hence, these organisms are limited in their tolerance to such environmental conditions (Mangum and Van Winkle, 1973; Pamatmat, 1978; Herreid, 1980).

The most common method used to determine an organism's metabolic rate is to measure its oxygen consumption rate (Burggren and Roberts, 1991). Such respirometric measurements are relatively easy to make, but determine only the aerobic metabolic rate of the organism. If the organism has the capacity to utilize anaerobic pathways, this method will underestimate the total metabolic rate. An alternative approach is to measure an organism's heat production using calorimetric techniques. Calorimetry can be used to measure the contribution of both aerobic and anaerobic pathways to the organism's total metabolism.

Several investigators have used respirometric and calorimetic techniques, either simultaneously or in parallel, to analyze the metabolic response of many aquatic invertebrates exposed to decreasing environmental oxygen tensions (eg. Pamatmat, 1978; Gnaiger and Staudigl, 1987; Doeller et al, 1990; Wang and Widdows, 1993). Such techniques can demonstrate several metabolic phenomena such as the partitioning of metabolic rates into aerobic and anaerobic components, the critical oxygen partial pressures where metabolism shifts from aerobic to anaerobic pathways, and the net energy produced when utilizing anaerobic pathways (Herreid, 1980; Gnaiger, 1983; Gnaiger et al, 1989). In this study, heat and oxygen flux of juvenile *C. quadricarinatus* were measured in parallel to examine the metabolic response of this crayfish when exposed to normoxic, hypoxic, and anoxic conditions. Specifically, we wanted to determine critical oxygen tensions (P_c) where oxygen consumption rate is limited by decreasing environmental p_{O_2} and to determine whether anaerobic pathways could be used by this crayfish to sustain metabolic rate during environmental anoxia.

MATERIALS AND METHODS

Cherax quadricarinatus

Newly hatched juvenile crayfish (9-15 mm total length, 10-50 mg fresh weight) were obtained from the aquaculture facility at the University of Alabama at Birmingham. Broodstock crayfish were maintained in 2.5 m x 0.6 m raceways at 28°C with associated

recirculating biofiltration systems. Mated females were held individually to avoid stress during the egg development period. After hatching, juvenile crayfish were placed individually in polystyrene bowls containing 200 ml of aerated tapwater and fed *ad libitum*. Juveniles were monitored for molts and/or deaths for at least 1 week prior to being used in experiments to ensure that the crayfish were in good health. Prior to any metabolic measurments, juveniles were starved for approximately 24 hr to minimize the metabolic condition of the crayfish as a result of their nutritional state.

Respirometry

Oxygen flux of individual crayfish was measured at 28°C using a closed-chamber temperature-controlled polarographic respirometer with dual pyrex chambers, titanium stoppers, and magnetic stirrers (Oxygraph 67097, Cyclobios-Paar, Austria). Each chamber was also equipped with a side view port which allowed limited visual observations of crayfish during experiments. Prior to crayfish introduction, the respirometry chamber was filled with 3-5 ml filtered (0.22 μm) water, stirred (500 rpm), and aerated until air saturated. Experiments were initiated by placing an individual crayfish on a mesh screen suspended above the stirring apparatus located in the bottom of the chamber. Once sealed in the respirometer chamber, crayfish were allowed to consume oxygen until tensions approached zero. To account for the possible introduction of foreign oxygen consumers, such as bacteria in the gills of the crayfish, blank oxygen consumption rates were measured before and after each experimental run. Oxygen consumption rates were recorded and analyzed using PC-based software designed specifically for the Oxygraph respirometer. Oxygen consumption rates for juvenile crayfish in this study are abbreviated as $\dot{n}O_2$ and reported as pmol O_2/(s*mg fresh weight).

Calorimetry

Heat flux of juvenile crayfish was measured using a calorimeter (Thermal Activity Monitor 2277, ThermoMetric, Sweden) as described by Gnaiger (1983) and Gnaiger et al.

(1989). Individual crayfish were placed in a stirred (160 rpm) 5 ml perfusion calorimetry cell containing filtered (0.22 μm) water of varying oxygen tensions. The perfusion rate into the calorimeter cell was 7.5 μl s^{-1}. Heat flux was determined for individual crayfish exposed to water equilibrated at 20.7, 10.4, and 5.2 kPa p_{O_2} (100, 50, and 25% air saturation, respectively), and anoxia. Actual p_{O_2} within the perfusion chamber ranged from 18.1 to 20.7, 7.8 to 10.4, and 2.5 to 5.2 kPa, respectively, as estimated using previous respirometer measurements of oxygen consumption rates. Inflow oxygen tensions of the test solutions were monitored continuously during the experiments. Experiments were initiated by perfusing the calorimetry chamber with 100% air-saturated water to obtain normoxic heat flux. Once heat flux stabilized, the inflow reservoir was bubbled with argon gas until achieving desired oxygen tensions. Step changes in p_{O_2} within the calorimeter cell occurred in approximately 20-30 min. Following several hours of exposure to test oxygen tensions, inflow water was re-aerated to 100% air saturation to allow crayfish to return to normoxic conditions. Blank heat dissipation rates were again recorded before and after each experiment. Heat dissipation rates are abbreviated as $-\dot{Q}$ and reported as μW/mg fresh weight. Once determined, values of oxygen and heat flux from parallel experiments at similar oxygen tensions were then used to calculate calorimetric/respirometric (C/R) ratios.

Data Analysis

Data are represented as mean ± standard error of the mean (error bars in figures). Number of individuals is given in parentheses. Heat dissipation data were statistically compared using the one-tailed paired Student's t-test since it was assumed that heat dissipation rates would likely decrease, not increase, as p_{O_2} decreased within the calorimeter.

RESULTS

Oxygen flux

Oxygen consumption rates of juvenile *Cherax quadricarinatus* at 28°C are shown as a function of p_{O_2} in Figure 1. Oxygen flux averaged 5.0 ± 0.4 (10) pmol O_2/(s*mg fresh weight) at normoxic oxygen tensions and remained nearly constant over the range of p_{O_2} from 20.7 down to approximately 4 kPa. At this rate of oxygen consumption, juvenile crayfish consumed and reduced p_{O_2} within the respirometer chamber to ~4 kPa typically within 20 min. At or below this critical p_{O_2} (P_c) of 4 kPa, oxygen flux decreased substantially, conforming to ambient oxygen tensions, until reaching zero. The time required for juvenile crayfish to reduce p_{O_2} from 4 to 0 kPa was approximately 10 min.

When initially placed in the respirometer chambers, crayfish exhibited rapid tail-flipping movements. Within a few minutes, however, most crayfish ceased this behavior and remained still throughout the duration of the experiment. As oxygen tensions inside the chamber decreased, no apparent changes were noted in crayfish behavior, even as tensions reached critically low p_{O_2}. Precise observations of crayfish behavior as oxygen tensions decreased, however, were not possible because of the limited view of the inside of the respirometry chamber.

Heat flux

Heat dissipation rates of juvenile *Cherax quadricarinatus* are shown as a function of p_{O_2} in Figure 2. When exposed to water near 100% air saturation (18.1 to 20.7 kPa p_{O_2}), heat flux averaged -2.2 ± 0.2 (9) μW/mg fresh weight (Fig. 2a, 2b,and 2c). At these normoxic conditions, the ratio of the amount of heat dissipated per amount of oxygen consumed, called the calorimetric/respirometric (C/R) ratio, averaged -440 kJ/mol O_2. When crayfish were exposed to water approaching 50% air saturation (7.8 to 10.4 kPa p_{O_2}), heat flux did not change significantly from normoxic heat flux values (Fig. 2a). When crayfish were exposed to water approaching 25% air saturation (2.5 to 5.2 kPa

Figure 1. Oxygen flux ($\dot{n}_{O_2}$) of juvenile *Cherax quadricarinatus* as a function of p_{O_2}. Arrow indicates critical oxygen tension (P_c). Each data point represents the average of 10 individuals.

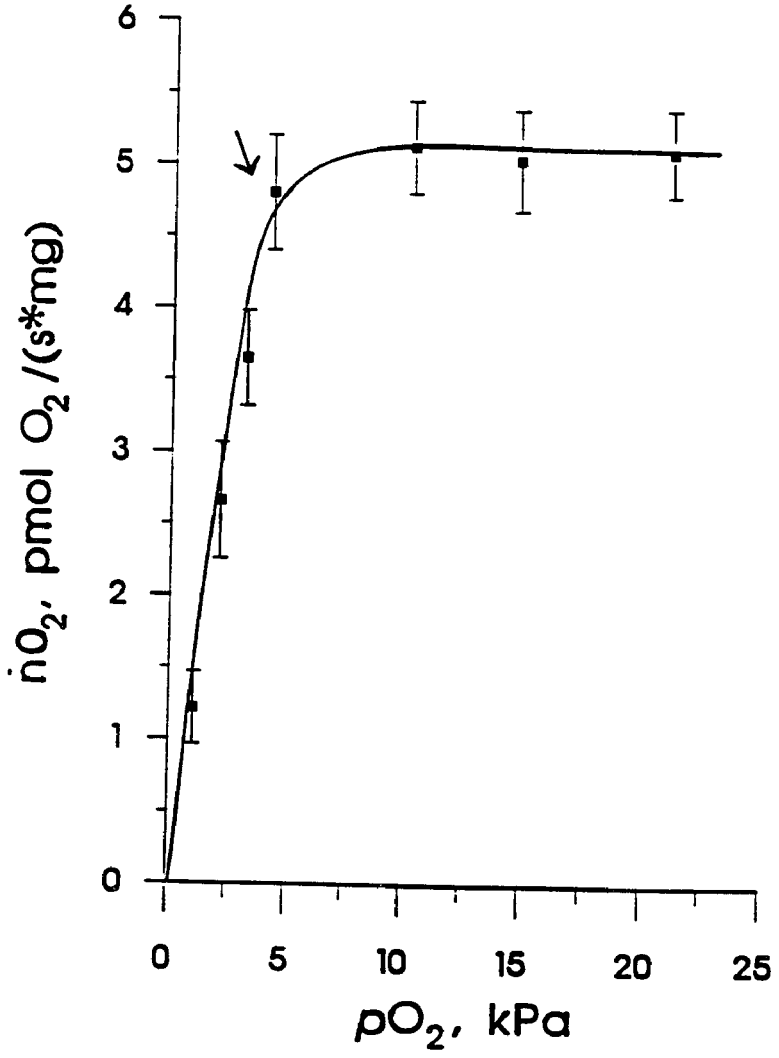
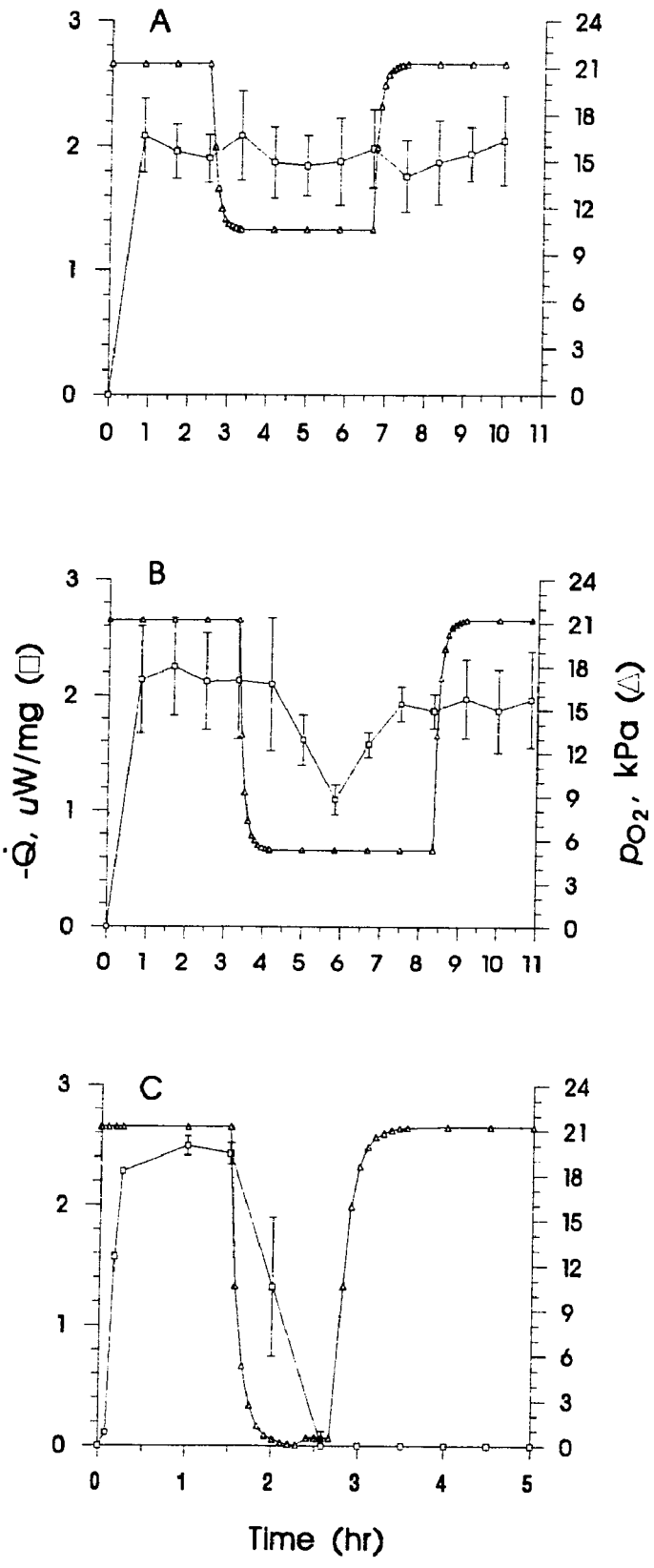

6
5
4
3
2
1
0
0
5
10
15
20
25
$\dot{n}O_2$, pmol O_2 /(s*mg)
pO_2, kPa

Fig. 2. Heat flux ($-\dot{Q}$) of juvenile *Cherax quadricarinatus* as a function of p_{O_2}. The p_{O_2} reported on the abscissa was measured in the inflow water of the perfusion chamber. **A:** Heat flux of animals exposed to water at 100% and 50% air saturation (18.1 to 21.7 and 7.8 to 10.4 kPa p_{O_2}, respectively). **B:** Heat flux of animals exposed to 100% and 25% air saturation (18.1 to 21.7 and 2.5 to 5.2 kPa p_{O_2}, respectively). **C:** Heat flux of animals exposed to 100% air saturation (18.1 to 21.7 kPa p_{O_2}) and anoxic conditions. Data points in A, B, and C are the average of 3 individuals in each experiment.


A
B
C
-Q̇, uW/mg (□)
pO2, kPa (△)
Time (hr)

p_{O_2}), heat flux decreased significantly to -1.1 ± 0.4 (3) μW/mg fresh weight ($p = 0.035$), about 50% of normoxic values, in approximately 2 hr. Within 4 hr of exposure to 25% air saturation conditions, however, heat flux returned to normoxic values (Fig. 2b). When crayfish were exposed to anoxic water (0 kPa p_{O_2}), heat flux decreased to zero within about 1 hr (Fig. 2c). Subsequent exposure of these crayfish to inflow water near 100% air saturation did not elicit a return to normoxic heat flux. Indeed, juvenile crayfish did not survive short term (1-2 hr) exposure to anoxic conditions.

DISCUSSION

The Australian crayfish, *Cherax quadricarinatus*, must have the ability to maintain metabolic functions to survive when exposed to hypoxic or anoxic environmental conditions. In this study, within the p_{O_2} range from 20.7 to 5.2 kPa, juvenile crayfish maintained oxygen flux at near normoxic values, demonstrating the ability of these animals to regulate oxygen consumption rate, hence aerobic metabolism, over a wide range of oxygen tensions. At environmental p_{O_2} below ~ 4 kPa, however, oxygen consumption rates conformed to decreasing p_{O_2}. Morrissy et al. (1984) observed a similar metabolic response in the adult Australian freshwater crayfish, *Cherax tenuimanus* (marron) and *Cherax destructor* (yabbie). Normoxic oxygen flux for these animals was maintained over the range of environmental p_{O_2} from air saturation down to approximately 2 to 8 kPa p_{O_2}. Below their respective critical oxygen tensions (P_c), respiration rates of these crayfish conformed to ambient oxygen tensions.

Critical oxygen tensions and oxygen consumption rates for *Cherax quadricarinatus* and several other burrowing and non-burrowing crustacean species are shown in Table 1. The low P_c value for *C. quadricarinatus* suggests that this animal can maintain aerobic metabolism in many habitiats, such as deep burrows, despite fluctuations in environmental p_{O_2}. Conversely, the P_c value for *Homarus vulgaris*, a non-burrowing crustacean that inhabits areas with relatively constant oxygen tensions, is somewhat higher. Critical oxygen tensions thus appear to correlate with the burrowing habits of the

Table 1. Critical oxygen tensions (P_c) and oxygen consumption rates ($\dot{n}_{O_2}$) for various crustacean species.

Burrowing species	*Pc* (kPa)	$\dot{n}_{O_2}$[a]	Temp.(°C)	Author
Uca pugnax	2	c	27	Pamatmat, '78
Cherax destructor	2-5[b]	2[b]	25	Fradd, '74
Procambarus simulans	3-5[b]	1[b]	23-24	Larimer and Gold, '61
Cherax quadricarinatus	~4	5	28	this study
Orconectes virilis	4-5[b]	0.1-0.4[b]	18-21	McMahon et al., '74
Cherax tenuimanus	5-8[b]	2[b]	25	Morrissy, '84
Non-burrowing species				
Macrobrachium rosengergii	5	3	28	Spottes, '83
Homarus vulgaris	7[b]	0.3[b]	15	Butler et al., '78

[a] reported as pmol O_2/(s mg fresh weight)
[b]estimated from published data
[c]reported as 0.13 ml/hr/animal

animal: non-burrowers such as *Homarus vulgaris* and weak burrowers such as *Cherax tenuimanus* exhibiting higher P_c values than moderate burrowers such as *C. quadricarinatus* or strong burrowers such as *Uca pugnax*. Although critical oxygen tensions (P_c) for each of the burrowing and non-burrowing groups of crustaceans are conserved somewhat, oxygen consumption rates vary by an order of magnitude, possibly reflecting differences among species as influenced by experimental temperature rather than their burrowing habits (Table 1). Alternatively, ontogenetic differences (Mangum and Van Winkle, 1973; Herreid, 1980) or gill morphology differences (Morrissy et al., 1984) could attribute to the variabilities observed in oxygen consumption rates.

Because the respirometry measurements in this study were made using a closed chamber, it is likely that p_{CO_2} increased during the experiments (in previous experiments, p_{CO_2} increased from 0 to 1.5 kPa and pH decreased from 7.7 to 7.4 as oxygen tensions decreased within a closed chamber; unpublished data). In crustaceans, hemocyanin oxygen affinity typically increases as p_{CO_2} increases, leading to a reverse Bohr shift (Morris et al, 1985; Mangum and Burnett, 1986). Crustacean hemocyanin oxygen affinity, however, typically decreases as pH decreases, exhibiting a classic positive Bohr shift (for review, see Burggren and Roberts, 1991). Similarly, a positive Bohr shift has been decribed for hemocyanin isolated from the Australian crayfish, *Cherax destructor*, in the pH range from 6.8 to 7.8 (Jeffrey and Treacy,1980). Since increased p_{CO_2} and decreased pH have opposite effects, the hemocyanin oxygen affinity of *C. quadricarinatus* may have changed little during the closed-chamber respirometric measurements.

Juvenile *Cherax quadricarinatus* maintained normoxic heat dissipation rates over the range from 18.1 to 20.7 and 7.8 to 10.4 kPa p_{O_2}. The substantial drop in heat flux and subsequent return to normoxic flux observed over the range from 2.5 to 5.2 kPa p_{O_2} suggests that this crayfish may compensate to maintain metabolic rate at p_{O_2} near the P_c. Previous studies have demonstrated several behavioral, physiological, and cellular compensatory mechanisms by which crustaceans adjust and maintain metabolic rate at

critically low p_{O_2}. The Malaysian freshwater prawn *Macrobrachium rosenbergii* induced an increase in hemolymph pH via hyperventilation when exposed to approximately 5 kPa p_{O_2} (Mauro and Malecha, 1984). The higher hemolymph pH increased the oxygen affinity of hemocyanin via a Bohr shift, thus enabling the prawn to continue extracting oxygen in an oxygen poor environment. In the crayfish *Orconectes virilis*, exposure to environmental p_{O_2} below 4 kPa increased cardiovascular pumping, resulting in hyperirrigation of gill tissues (McMahon et al., 1974). Initially, this increased cardiovascular activity resulted in slightly higher oxygen consumption rates as a result of increased metabolic activity. However, following several days of exposure to hypoxic conditions, oxygen flux returned to near-normal values as cardiovascular pumping decreased while brachial irrigation rates remained above pre-hypoxic levels (McMahon et al., 1974). Lastly, the anaerobic end-product lactate, at increased levels in the hemolymph, allosterically increased hemocyanin oxygen affinity in the crayfish, *Austropotamobius pallipes* (Morris et al., 1986). We suggest that in juvenile *C. quadricarinatus*, aerobic compensatory mechanisms similar to these could result in the recovery of metabolic rates during exposure to critically low p_{O_2}.

The normoxic CR ratio measured in this study is within the theoretical oxycaloric equivalent as observed for a variety of animals at normoxic conditions (- 430 to - 480 kJ/mol O_2), indicating that the metabolism of *Cherax quadricarinatus* is fully aerobic and dissipative (Gnaiger and Staudigl, 1987). Because oxygen consumption rates at p_{O_2} less than air saturation were not measured at steady state, CR ratios at low p_{O_2} could not be determined. Therefore, no conclusions can be made concerning the ability of these juvenile crayfish to utilize anaerobic pathways to maintain metabolic rates during hypoxic stress. Metabolic rates were, however, maintained after a 2 hr period in crayfish exposed to critically low p_{O_2}, suggesting that anaerobic pathways may have been induced and contribute to the maintenance of metabolic rate. Juvenile crayfish, nonetheless, could not maintain heat dissipation rates and did not survive short periods of anoxic stress,

suggesting that anaerobic pathways contribute little or nothing toward maintaining metabolic rate during environmental anoxia.

In summary, juvenile Australian crayfish *Cherax quadricarinatus* demonstrated the ability to regulate and maintain oxygen and heat flux over a wide range of environmental P_{O_2} from air saturation down to approximately 4 kPa. This ability to maintain metabolic rate at fluctuating environmental P_{O_2} enables the crayfish to survive in oxygen-poor environments such as deep burrows. At about 4 kPa P_{O_2}, however, oxygen flux in this crayfish begins to conform to decreasing P_{O_2}. In the hypoxic range from 2 to 5 kPa P_{O_2}, near the P_c, heat flux of this crayfish decreased and then returned to normoxic values, indicating the presence of compensatory mechanisms to maintain metabolism. The contributions from aerobic and/or anaerobic metabolic pathways that allow the maintenance of metabolic rate at hypoxic conditions are not fully understood. When environmental oxygen tensions fall to zero, however, heat flux decreases substantially until reaching zero, indicating limited ability for anaerobic pathways to sustain metabolism in this crayfish during anoxia. The anaerobic pathway contribution, if any at all, to the total metabolic rate in this crayfish during hypoxic or anoxic stress, however, could not be determined. Future studies evaluating changes in oxygen and heat flux that occur at steady state environmental P_{O_2} from 0 to 5 kPa could determine the compensatory mechanism(s) by which metabolism is maintained by this crayfish at critically low oxygen tensions and whether anaerobic pathways are utilized in this compensatory response.

ACKNOWLEDGMENTS

We thank Mr. Nick Chacos (Allied Chemical Technologies, Inc., Clifton, N.J.) for the use of the ThermoMetric calorimeter, and Sabine Piller for her assistance with respirometry measurements. This research was supported in part by NSF IBN9118048 to JED and DWK and by the Environmental Applied Aquaculture Institute.

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GROWTH, OXYGEN CONSUMPTION, AND ENERGY UTILIZATION IN JUVENILE AUSTRALIAN CRAYFISH, *Cherax quadricarinatus*, AT DIFFERENT TEMPERATURES AND SALINITIES

Authors: Mark E. Meade
Jeanette E. Doeller
David W. Kraus
Stephen A. Watts

To be submitted to: Physiological Zoology

ABSTRACT

Growth (weight gain) and oxygen consumption rates were examined in juvenile Australian crayfish, *Cherax quadricarinatus*, exposed to different temperatures or salinities. Weight gain, molting frequency, and survival of juveniles were temperature dependent. Maximal weight gain and molting frequencies were observed at 28°C. Above or below this temperature, weight gain was reduced. Maximal survival was observed over the temperature range of 24 - 30°C. Oxygen consumption rates of juveniles were temperature dependent; Q_{10} values averaged 2.44. Weight gain, molting frequency, and survival of juveniles were also salinity dependent. Maximal weight gain and molting frequencies were observed at salinities of 0 and 5 ppt. Above 5 ppt, weight gain was reduced. Survival was reduced at salinities above 0 ppt. Oxygen consumption rates of juveniles were not salinity dependent and did not differ significantly at salinities ranging from 0 to 20 ppt. Growth and oxygen consumption rate data were used to calculated growth efficiencies. Growth efficiencies were used to estimate the amount of energy used for growth processes relative to overall metabolic processes at the different temperatures and salinities. Growth efficiencies were highest at 24°C. Above or below 24°C, growth efficiencies decreased. We suggest that individuals cultured at 24°C were not temperature stressed, and were using energy efficiently despite exhibiting sub-maximal weight gain. We also suggest that at temperatures above and below 24°C, temperature stress increased the energy required for maintenance processes and limited the energy available for growth processes. Growth efficiencies were also highest at 0 and 5 ppt salinity. Above 5 ppt, growth efficiencies were reduced. We hypothesize that the energetic demands for osmotic and ionic regulation may have increased thus decreasing metabolic energy available for growth at the higher salinities. The ability to maintain molting frequency and maximal growth over a narrow range of environmental temperatures and salinities limits the culture of this species in areas where changes in climate are frequent.

INTRODUCTION

The freshwater Parastacid, *Cherax quadricarinatus*, is a large crayfish found in many streams and rivers throughout the northern regions of Australia. Populations inhabiting the inland and coastal areas of northern Australia can be exposed to substantial fluctuations in temperature as the seasons change (Reik, 1972). In addition, crayfish in both regions can be exposed to saline waters during certain times of the year (Bayly and Williams, 1973). Based upon their widespread distribution, *C. quadricarinatus* apparently have the ability to tolerate a range of different environmental conditions. Little information exists, however, concerning the physiological ecology of this crayfish species, particularly information on the effects of environmental temperature or salinity on growth and energy utilization.

In this study, we have examined parameters of growth and survival of juvenile crayfish cultured at various temperatures and salinities. The specific aims of this study were to (1) determine weight gain, molting frequency, and survival in response to different temperatures and salinities, and (2) relate differences in weight gain at different temperatures and salinities to energy use (as determined by metabolic rates).

MATERIALS AND METHODS

Culture experiments

Newly-hatched (10 ± 1 mg wet weight) juvenile *Cherax quadricarinatus* were randomly distributed and held individually in 10 cm diameter (bottom surface area ~ 80 cm^2) polystyrene bowls containing aerated tapwater (pH 7.5 ± 0.2, alkalinity 70 ± 5 mg/l, and hardness 300 ± 10 mg/l) (Masser et al., 1990). To determine the effects of temperature exposure, juvenile crayfish were cultured at temperatures of 16, 18, 20, 22, 24, 26, 28, 30, and 32 ± 0.5°C. Preliminary experiments have shown that, at temperatures below 16 or above 32°C, juvenile mortality approaches 100% in less than 2 weeks. A total of 30 juvenile crayfish (10 siblings from each of 3 broodstock females) were used for

each temperature treatment. The juvenile crayfish were cultured in an environmentally-controlled chamber using daylight fluorescent lighting on a 12:12 photoperiod and fed *ad libitum* AB crayfish feed (UAB Research Foundation), a diet previously determined as suitable for the growth and survival of *Cherax quadricarinatus* juveniles (Meade and Watts, in review). Uneaten food was removed and replaced daily with fresh food. Culture water was replaced every other day. Ammonia and nitrites were monitored daily in all containers using colorimetric test kits (Fritz Aquaculture Inc.).

To determine the effects of salinity exposure, newly-hatched juvenile crayfish were cultured at salinities of 0, 5, 10, 15, 20, 25, and 30 ppt. A total of 40 juvenile crayfish (10 siblings from each of 4 broodstock females) were used for each salinity treatment. Juveniles were cultured, as described above, in an environmentally-controlled chamber at $27 \pm 0.5^{o}C$ using daylight fluorescent lighting on a 12:12 photoperiod. Experimental saltwater was made by adding artificial sea salts (Tropic Marin, Germany) to aerated tapwater to obtain the desired test concentrations. The salinity test solutions were adjusted, as necessary, to copy those water quality characteristics of solutions used in the temperature treatments. To minimize stress, juveniles were placed in test solutions of increasing salinity (5 ppt increments) for 30 min intervals until reaching the desired test salinity (Buikema et al., 1982). The juvenile crayfish were fed *ad libitum* AB crayfish feed. Food and water changes, as well as monitoring of ammonia and nitrites, were made as described previously.

Juvenile crayfish in the temperature and salinity treatments were cultured for 70 days. All individuals were examined twice daily for molts and/or mortalities. Individual crayfish were measured at the end of the 70 day culture period to determine weight gain (mg). Mean weight gain values for the different treatments were analyzed using one-way ANOVA and Tukey's multiple comparison test (Daniel, 1987). Survival data were arcsine transformed and analyzed using the Chi-square test. Alpha for statistical analysis was 0.05.

Oxygen consumption

Oxygen consumption rates were measured at the end of the 70 day culture period using a minimum of 5 juvenile crayfish from each of the temperature or salinity experiments. Oxygen consumption rates were measured using a closed-chamber, temperature-controlled polarographic respirometer (model 67097, Cyclobios-Paar, Austria) as described by Meade et al. (1994). Briefly, before placing the crayfish in the respirometer, the holding chamber was filled and equilibrated with filtered (0.22 μm) water of the desired temperature or salinity, stirred (500 rpm), and aerated until air saturated (~ 21 kPa). Once placed in the respiratory chamber, crayfish were allowed to consume oxygen until tensions within the chamber approached zero (~ 1 to 2 kPa). Blank respirometry measurements were made before and after the crayfish was in the chamber. Differences in before and after blank respiratory measurements were attributed to microbial contamination. Linear changes in blank oxygen consumption rates were averaged and subtracted from oxygen consumption rates determined for crayfish. Oxygen consumption rates were recorded using Datgraf analysis software v 2.1 (Cyclobios, Innsbruck, Austria). Oxygen rates are abbreviated as n_{O_2}, and reported as pmol O_2/(s*mg wet weight) throughout the paper and in all figures. Mean oxygen consumption rates for the different treatments were compared using the unpaired t-test (Daniel, 1987). Alpha for statistical analysis was 0.05. Q_{10} values were calculated using formulas described by Burggren and Roberts (1991).

Apparent growth efficiency (APE)

Apparent growth efficiencies for the different temperature and salinity treatments were calculated using a modified equation (1) of Battley (1987). Mean oxygen consumption rates for individuals at normoxic oxygen tensions (16 kPa) were used in all calculations.

$$\text{AGE} = \frac{\text{energy conserved (out)}}{\text{energy consumed (in)}} = \frac{\text{weight gain}}{\text{weight gain + respiration rate}} \qquad (1)$$

Weight gain and respiratory values were converted to caloric equivalents before use in the calculations. Weight gain was converted to caloric values using the estimated conversion factor of 81.51 kcal/100 g weight (LA Crawfish Board). Respiration rates were converted to caloric values using the oxycaloric equivalent of 440 kJ/mol O_2 previously determined (Meade et al., 1994).

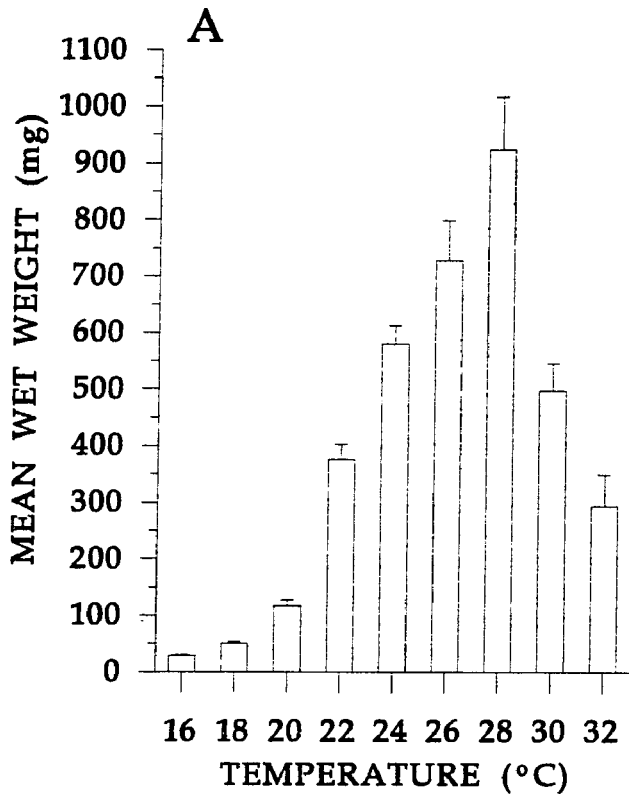
RESULTS

Culture experiments

Weight gain of the juvenile crayfish was temperature dependent (Fig. 1a). After 70 days, maximal weight gain was observed at 28°C with individuals averaging 923 ± 93 mg wet weight. At temperatures above or below 28°C, weight gain was significantly reduced, with individuals averaging 29.0 ± 1, 49.4 ± 4, 115.5 ± 10, 375.2 ± 26, 579.0 ± 32, 726.3 ± 71, 496.1 ± 49, and 292.5 ± 55 mg wet weight at 16, 18, 20, 22, 24, 26, 30, and 32°C, respectively. The maximal number of molts (~ 7) was observed at 28°C (Table 1). At temperatures above or below 28°C, the number of molts observed was reduced. Survival was 90% or higher in the temperature range of 24 - 30°C (Fig.1b). Survival above or below this temperature range was significantly reduced.

Similarly, weight gain of juvenile crayfish was salinity dependent (Fig. 2a). At salinities of 0 and 5 ppt, no significant differences were observed in weight gain, with individuals averaging 970 ± 64 and 997 ± 98 mg wet weight, respectively. At salinities of 10, 15, and 20 ppt, weight gain was significantly reduced, with individuals averaging 683 ± 94, 331 ± 66, and 232 ± 51 mg wet weight, respectively. Individuals exposed to 25 and 30 ppt died within 48 hr of initial exposure, hence, no weight gain data for these treatments are reported. The maximal number of molts (~ 7) was observed at 0 and 5 ppt (Table 2). At salinities above 5 ppt, the number of molts observed was reduced. Survival was the highest in individuals exposed to 0 ppt (95%). At 5, 10, 15, and 20 ppt, survival was significantly reduced (75, 66, 59, and 41%, respectively) (Fig. 2b).

Figure 1. Final mean wet weights (A) and percent survival (B) of newly-hatched juvenile *Cherax quadricarinatus* cultured for 70 days at different temperatures. n = 30 crayfish/treatment at the beginning of the experiments. (Initial weight ~10 mg/individual for all treatments)


A
MEAN WET WEIGHT (mg)
1100
1000
900
800
700
600
500
400
300
200
100
0
16 18 20 22 24 26 28 30 32
TEMPERATURE (°C)

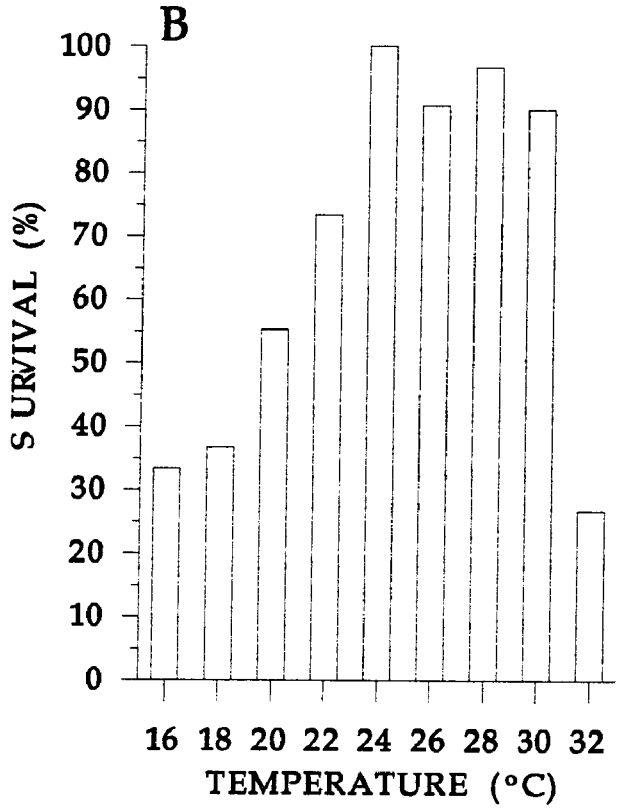
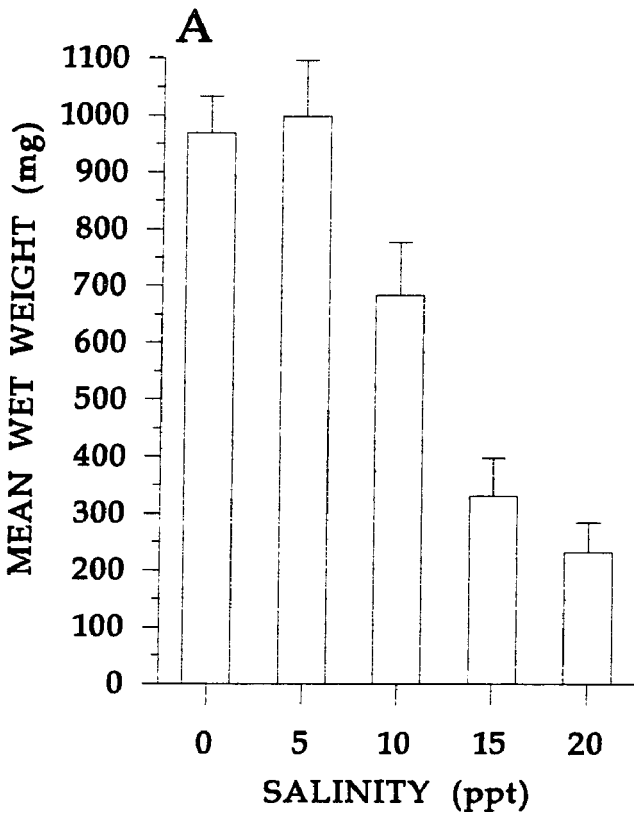

B
SURVIVAL (%)
100
90
80
70
60
50
40
30
20
10
0
16 18 20 22 24 26 28 30 32
TEMPERATURE (°C)

Table 1. Mean number of molts ± se for juvenile crayfish surviving 70 days of culture at different temperatures.

Temperature (°C)	n	# molts
16	10	1.8 ± 0.4
18	11	2.3 ± 0.4
20	17	3.5 ± 0.1
22	22	4.0 ± 0.2
24	30	4.5 ± 0.2
26	27	5.4 ± 0.2
28	29	6.8 ± 0.2
30	27	3.6 ± 0.3
32	8	3.0 ± 0.3

Figure 2. Final mean wet weights (A) and percent survival (B) of newly-hatched juvenile *Cherax quadricarinatus* cultured for 70 days at different salinities. n = 40 crayfish/treatment at the beginning of the experiments. (Initial weight ~10 mg/individual for all treatments)


A
MEAN WET WEIGHT (mg)
1100
1000
900
800
700
600
500
400
300
200
100
0
0
5
10
15
20
SALINITY (ppt)

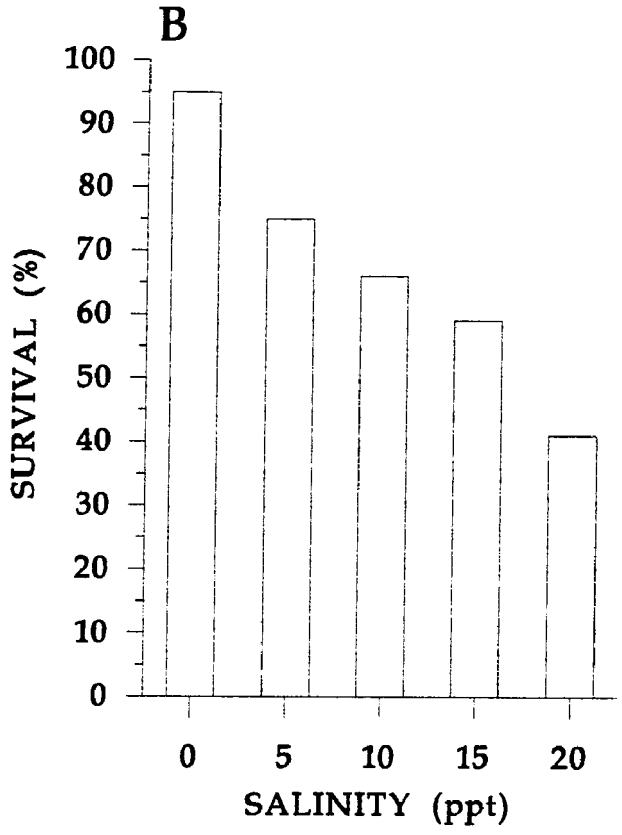

B
SURVIVAL (%)
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
SALINITY (ppt)

Table 2. Mean number of molts ± se for juvenile crayfish surviving 70 days of culture at different salinities.

Salinity (ppt)	n	# molts
0	38	6.8 ± 0.5
5	30	6.5 ± 0.7
10	27	5.2 ± 0.3
15	24	3.6 ± 0.2
20	16	2.7 ± 0.5

Oxygen consumption

Mean oxygen consumption rates ranged from 2.13 to 9.06 pmol O_2/s*mg wet weight over the temperature range of 16 to 32°C (Fig. 3). Over this temperature range, calculated Q_{10} values averaged 2.44 for every 4°C increase in temperature (Table 3). For all temperature treatments, oxygen consumption rates were regulated over the p_{O_2} range from fully air saturated conditions (~ 21 kPa) to approximately 20% of air saturated conditions (~ 4 kPa) (data not shown). Below 4 kPa p_{O_2}, oxygen consumption rates conformed to decreasing oxygen tensions.

Mean oxygen consumption rates ranged from 5.3 to 6.0 pmol O_2/s*mg wet weight over the salinity range of 0 to 20 ppt and were not significantly different (Fig. 4). For all salinity treatments, oxygen consumption rates were regulated over the p_{O_2} range from fully air saturated conditions to approximately 20% of air saturated conditions (~ 4 kPa) (data not shown). Below 4 kPa p_{O_2}, oxygen consumption rates conformed to decreasing oxygen tensions.

Apparent growth efficiency

For juvenile crayfish exposed to the different temperatures, the highest AGE was observed at 24°C (Fig. 5). Above and below this temperature, AGE decreased. For juvenile crayfish exposed to the different salinities, the highest AGE was observed at 0 and 5 ppt (Fig. 6). At salinities above 5 ppt, AGE decreased until reaching a value near zero at 25 ppt.

DISCUSSION

Culture experiments

Newly-hatched juvenile *Cherax quadricarinatus* were able to grow (increase in weight) and survive at environmental temperatures ranging from 16 to 32°C. Jones (1988) observed a similar tolerance range of environmental temperatures for larger juvenile *C. quadricarinatus*, reporting growth and survival between 20 and 34°C. Comparatively, Morrissy (1990) reported growth and survival of *C. tenuimanus* at

Figure 3. Oxygen consumption rates of juvenile *Cherax quadricarinatus* determined at normoxic conditions (~16 kPa) and measured at the temperatures which they were cultured. n = 5 crayfish/treatment.

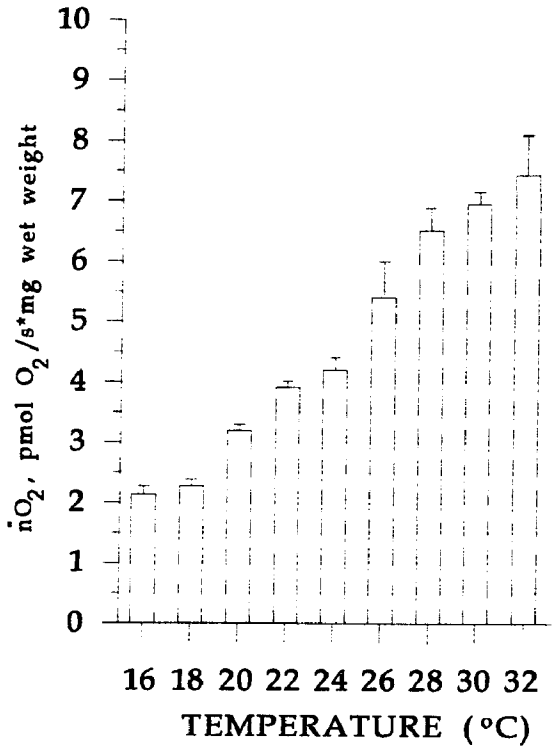

10
9
8
7
6
5
4
3
2
1
0
$\dot{n}O_2$, pmol O_2/s*mg wet weight
16 18 20 22 24 26 28 30 32
TEMPERATURE (°C)

Table 3. Q_{10} values for juvenile *C. quadricarinatus* at 4°C intervals over the temperature range of 16 - 32°C.

Temperature (°C)	Q_{10}
16-20	2.73
18-22	3.87
20-24	1.99
22-26	2.26
24-28	3.00
26-30	1.87
28-32	1.39
Avg.	2.44

Figure 4. Oxygen consumption rates of juvenile *Cherax quadricarinatus* determined at normoxic conditions (~16 kPa) and measured at the salinities which they were cultured. n = 5 crayfish/treatment.

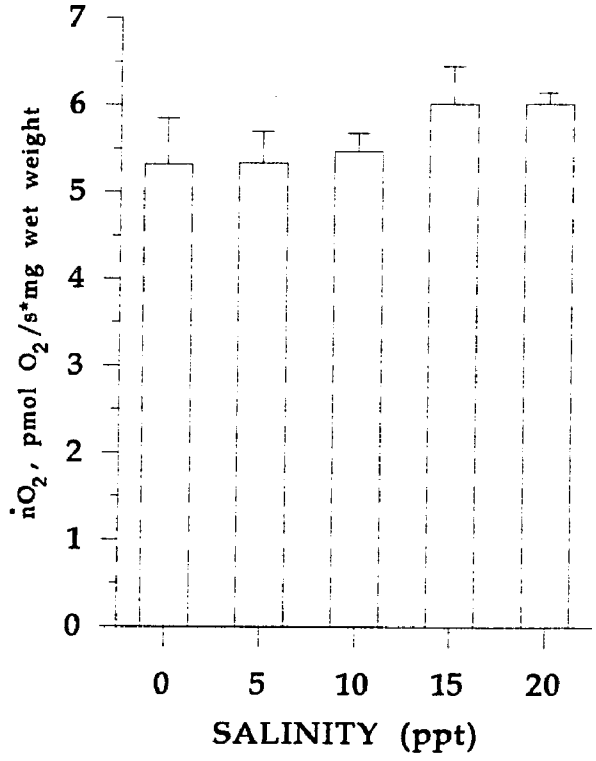

7
6
5
4
3
2
1
0
$\dot{n}O_2$, pmol O_2/s*mg wet weight
0
5
10
15
20
SALINITY (ppt)

Figure 5. Apparent growth efficiencies (AGE) (dotted line) and final mean weights (solid line) of juvenile *Cherax quadricarinatus* cultured at different temperatures.

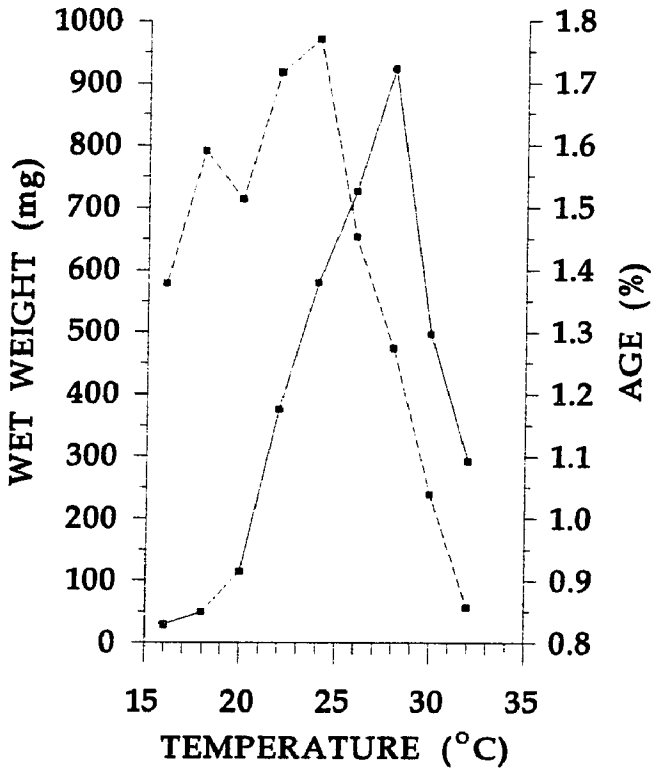
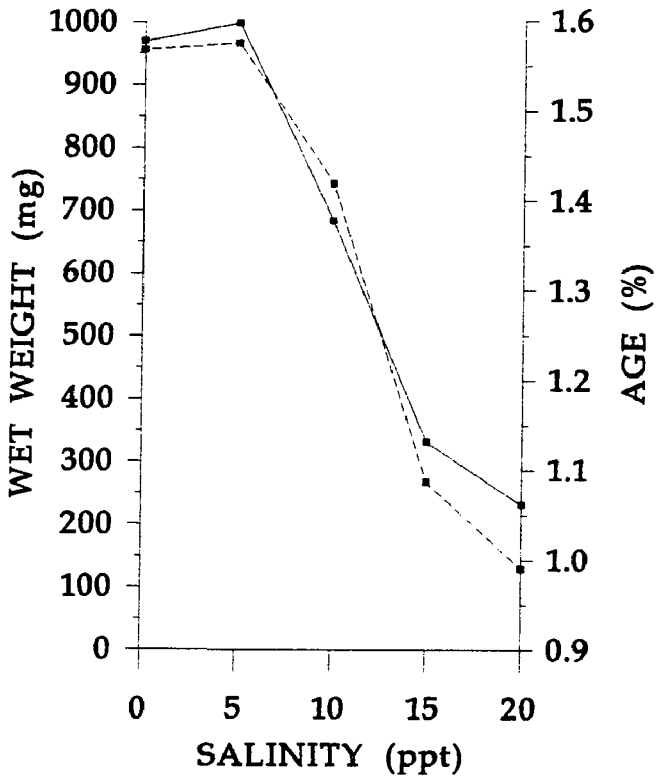

1000
900
800
700
600
500
400
300
200
100
0
WET WEIGHT (mg)
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
AGE (%)
15
20
25
30
35
TEMPERATURE (°C)

Figure 6. Apparent growth efficiencies (AGE) (dotted line) and final mean weights (solid line) of juvenile *Cherax quadricarinatus* cultured at different salinities.


1000
900
800
700
600
500
400
300
200
100
0
WET WEIGHT (mg)
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
AGE (%)
0
5
10
15
20
SALINITY (ppt)

environmental temperatures ranging from 13 to 30°C, and Mills (1986) reported growth and survival of *C. destructor* at environmental temperatures ranging from 15 to 36°C.

Although crayfish of the genus *Cherax* are considered freshwater species, salinities of many coastal and inland waters in Australia have been recorded as high as 6 ppt (Bayly and Williams, 1973). Investigators have suggested that *Cherax* spp. may have evolved mechanisms to tolerate saline waters. In this study, weight gain of juvenile *C. quadricarinatus* was not affected by salinities ranging from 0 to 5 ppt, however, above 5 ppt, weight gain and survival were significantly reduced. Mills and Geddes (1980) demonstrated that juvenile and adult *C. destructor* could tolerate salinities of 26 and 30 ppt, respectively, without significant mortality during 8 days of exposure. Mills and Geddes (1988) further observed positive weight gain in *C. destructor* at salinities up to 10 ppt; however, weight gain was reduced at salinities above 6 ppt. Many other crayfish species (e.g., *Procambarus clarkii*) can tolerate salinities equaling full strength seawater for short durations (for review, see Vernberg and Vernberg, 1983). Chronic exposure to saline waters, however, usually causes high mortalities. We suggest that weight gain and survival of *P. clarkii* and other crayfish species would also be reduced when cultured in saline waters above 5 ppt.

Molt intervals (a measure of molting frequency) and molt increments (the increase in size at each molt) can be related to weight gain observed in cultured crustaceans. Environmental factors such as temperature and salinity have been demonstrated to affect both molt intervals and molt increments of crustaceans (for review, see Hartnoll, 1982). For example, as temperature increased from 14 to 24°C, the molt interval of *C. tenuimanus* decreased, allowing for an increased number of molts and an increased weight gain (Morrissy, 1990). Molt increments, however, did not change substantially for *C. tenuimanus* over this temperature range. Likewise, as temperature increased from 16 to 28°C, the molt interval of juvenile *C. quadricarinatus* decreased, resulting in an increased number of molts and an increased weight gain. Molt intervals at "optimal" salinities have

also been demonstrated to decrease in duration and allow for increased molting in many crustacean species (for review, see Hartnoll, 1982). Molt increments among crayfish in the different temperature and salinity treatments in this study were not determined. In previous studies, however, diet has been shown to alter molt intervals as well as molt increments in cultured juvenile *C. quadricarinatus* (Meade and Watts, in review). We suggest that molt increments may have also changed and contributed to the observed differential weight gain of juvenile *C. quadricarinatus*.

Oxygen consumption

Oxygen consumption rates of juvenile *Cherax quadricarinatus* varied directly with environmental temperature. Q_{10} values (≥ 2) suggest that the metabolic rate of this species is temperature dependent over the range of 16 - 32°C. Other crustacean species show a similar response (for review, see Vernberg, 1983). For example, over the temperature range of 16 - 35°C, the crayfish *Orconectes immunis* and *O. nais* consume oxygen with an increase in temperature, with Q_{10} values of approximately 2. Oxygen consumption rates of juvenile *C. quadricarinatus* were not affected by salinity. For many crustacean species, fluctuations in environmental salinity can cause either an increase, decrease, or no change in oxygen consumption rate, depending on the species (for review, see Vernberg, 1983). The response observed for juvenile *C. quadricarinatus* is typical of a crustacean species which inhabits areas where abrupt changes in salinity can occur. The brine shrimp, *Artemia salina*, is an example of one such species (Kinne, 1964). Investigators hypothesize that the oxygen consumption rates of such species can be maintained because of efficient osmoregulatory processes.

Apparent growth efficiency

Metabolism provides the energy that is required to fuel life processes. All metabolic reactions, whether concerning the processes of growth, reproduction, or maintenance, can be closely related. Determining the contribution of metabolic energy utilized to perform particular physiological functions, however, can be a difficult task.

The concepts of compensation and adaptation nonetheless presuppose the potential variable utilization of metabolic energies (Sibly and Calow, 1986). Such subdivision can be useful in quantifying the relative proportion of metabolism allocated to different physiological functions (Hawkins and Bayne, 1992). Indeed, calorimetric measurements of heat dissipation, combined with measurements of total energy (caloric) content of soft tissues in the marine mollusk, *Mytilus edulis*, have been used to assess the relative allocation of metabolism for processes including maintenance, feeding, digestion/absorption, and growth (Widdows and Hawkins, 1989). Furthermore, studies examining the energetics of physiological processes in microorganisms have determined the efficiency of input energies for growth processes (for review, see Battley, 1987).

Previous studies using calorimetric and respirometric techniques have demonstrated that the metabolism of juvenile *Cherax quadricarinatus* is aerobically dissipative at normoxic conditions (Meade et al., 1994). In this study, we combined measurements of metabolic rate (total energy input) with measurements of weight gain (energy used for assimilation) to assess the relative proportion of metabolic energy allocated for growth processes in juvenile *C. quadricarinatus* cultured at different temperatures and salinities. Precise caloric values were not determined for the crayfish themselves, and estimates were used based on caloric values for crayfish tail meat. Nonetheless, our measurements could be used to infer the relative allocation of metabolic energies for growth processes. We hypothesized that individuals at optimal environmental conditions would have higher growth efficiencies than individuals at sub-optimal environmental conditions. We also suggest that comparisons of "growth efficiencies" of conspecifics cultured at different environmental conditions could be used to determine the "well being" of individuals at any one condition relative to another (i.e., stress vs. non-stress condition).

For individuals exposed to the different temperatures, the highest apparent growth efficiency was observed at 24°C and did not coincided with those individuals that

exhibited the highest weight gain (28°C). We suggest that higher weight gain at 28°C occurred because of the presence of unlimited food resources, despite a lack of efficiency for utilizing such resources. Food intake was not monitored in this study; however, casual observations indicated differential food intake at the different temperatures. Based on energetics of microbial growth at conditions of limiting and non-limiting food resources (Battley, 1987), we suggest that, at conditions of limited food resources, weight gain may have been higher in individuals cultured at 24°C .

For individuals exposed to the different salinities, the highest apparent growth efficiencies were observed at 0 and 5 ppt, and coincided with the highest weight gain observed. Previous reports suggest that reduced mortalities occur when *C. quadricarinatus* and *C. tenuimanus* are cultured in slightly saline waters (~ 100 ppm) (Rouse and Kartamulia, 1992; Anson and Rouse, 1994. Since salinities as high as 3 ppt have been recorded for many of the coastal and inland waters in Australia (Bayly and Williams, 1973), it is not surprising that juvenile *C. quadricarinatus* can grow at salinities up to 5 ppt. At salinities above 5 ppt, growth efficiencies decreased. Since metabolic rates (oxygen consumption rates) did not increase with increasing salinity, it is possible that the energetic demands for osmotic and ionic regulation increased (i.e., maintenance costs increased), thus decreasing metabolic energy available for growth. Alternatively, although not measured in this study, food intake may have decreased in individuals exposed to higher salinities, thus resulting in the observed reduced weight gain.

Cherax quadricarinatus can be classified as a eurythermal, mesohaline crayfish. Although this crayfish tolerates a diverse range of temperatures and salinities, a narrower range of temperatures and salinities exists where growth or energy utilization are optimized. The consequences of a lack of available energy for growth at sub-optimal temperatures and salinities can be illustrated further by the reduced ability to molt. Whether food intake is decreased, resulting in a lack of energy for growth, or energy is allocated for maintenance processes related to stress, the energy required to maintain molt

frequencies, and thus maximal growth, may not be available at certain temperatures or salinites. Since this species of crayfish has been demonstrated to have potential commercial value, culturists should be aware of the limiting effects of environmental temperature and salinity on growth, particularly in those areas where changes in environmental temperatures or salinities are prevalent or in situations where food resources are limiting.

ACKNOWLEDGMENTS

This study was supported in part by the Department of Biology at the University of Alabama at Birmingham and by NSF IBN9343430 to JED and DWK.

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TOXICITY OFAMMONIA, NITRITE, AND NITRATE TO JUVENILE AUSTRALIAN CRAYFISH, *Cherax quadricarinatus*

Authors: Mark E. Meade
Stephen A. Watts

To be submitted to: The Journal of Shellfish Research

ABSTRACT

Survival rates and metabolic (oxygen consumption) rates were examined in juvenile Australian crayfish, *Cherax quadricarinatus*, exposed to different concentrations of ammonia, nitrite, or nitrate. The relative tolerances of these crayfish to ammonia, nitrite, and nitrate are similar to those reported for other fish and crustacean species. No mortalities were observed in crayfish exposed to concentrations up to 25 mg/l total NH_3-N (0.54 mg/l un-ionized NH_3-N). Crayfish exposed to 50, 100, and 200 mg/l total NH_3-N (1.07, 2.14, and 4.28 mg/l un-ionized NH_3-N) survived an average of 40, 36, and 14 hr, respectively. Calculated LC_{50} values for 24, 48, and 96 hr were 94.3 ± 0.24, 76.3 ± 0.12, and 45.9 ± 0.25 mg/l total NH_3-N, respectively (2.02, 1.63, and 0.98 mg/l un-ionized NH_3-N). No mortalities were observed in crayfish exposed to concentrations up to 10 mg/l NO_2-N. Crayfish exposed to 25, 50, and 100 mg/l NO_2-N survived an average of 96, 22, and 5 hr, respectively. Calculated LC_{50} values for 24, 48, and 96 hr were 42.9 ± 0.22, 37.1 ± 0.16, and 25.9 ± 0.35 mg/l NO_2-N, respectively. No mortalities were observed in crayfish exposed to nitrate concentrations up to 1000 mg/l. Oxygen consumption rates of crayfish exposed to fresh water (controls) were regulated over the p_{O_2} range of 20 to 5 kPa. Oxygen consumption rates of crayfish exposed to ammonia or nitrate did not differ significantly from those of control crayfish over the same range of environmental p_{O_2}. Oxygen consumption rates of crayfish decreased immediately upon exposure to nitrite and were never recovered. At 10 kPa p_{O_2}, oxygen consumption rates of crayfish exposed to nitrite were approximately 50% of controls. In conditions of low environmental p_{O_2}, nitrite could substantially affect the survival of individuals.

INTRODUCTION

Ammonia (NH_3) comprises 40 to 90% of the nitrogenous excretion of crustaceans (Parry, 1960). Under aerobic conditions, ammonia can be oxidized by nitrifying bacteria (*Nitrosomonas* and *Nitrobacter* spp) to nitrite (NO_2^-)and nitrate (NO_3^-) (Sharma and Ahlert, 1977). Ammonia and nitrite are the most common pollutants found in intensively

managed aquaculture systems and, as concentrations increase, they can negatively affect the production of fish, crustaceans, and other aquatic organisms (Colt and Armstrong, 1981). Nitrate can also increase in aquatic systems to relatively high concentrations; however, it does not appear to directly affect the health of individuals (Russo, 1985).

For many of the commercial fish species, the lethal concentrations of ammonia, nitrite, or nitrate, including their potential mechanism(s) of toxicity, have been examined (for review, see Russo, 1985). Several investigators suggest that ammonia and/or nitrite directly affect the health of fish by decreasing the oxygen binding affinity of hemoglobin, causing a physiological hypoxia (Brockway, 1950; Russo, 1985). Other investigators suggest that ammonia and/or nitrite can directly affect the central nervous system of fish, causing a depletion of adenylates (Smart, 1978). Nitrate appears to affect the health of fish indirectly. For example, in many static systems, algal blooms, caused by elevated nitrates, can cause excessive eutrophication, resulting in environmental hypoxia.

Concentrations of ammonia and nitrite lethal to several crustacean species have also been determined. Knowledge of the potential mechanism(s) of toxicity of these compounds to crustaceans remains unclear. Despite conclusive evidence, some investigators suggest that ammonia and/or nitrite affect crustaceans in a manner similar to that observed in fish; by binding to hemocyanin and decreasing oxygen binding affinity (Sanders et al., 1992; Needham, 1961). Few, if any, investigators have examined the lethal concentrations of nitrate or its potential mechanism(s) of toxicity to crustaceans.

Australian freshwater crayfish of the genus *Cherax* are rapidly gaining attention among commercial culturists worldwide. Crayfish in this genera are typically fast growers, attain large sizes (up to several kg), and have multiple spawns annually (Semple et. al., 1995). One species, *Cherax quadricarinatus*, is gaining popularity among culturists in the southern latitudes of the US. Little information exists on the effects of water-born toxicants, such as ammonia, nitrite, or nitrate, on this culture species. Such information, however, is prerequisite to defining those conditions necessary for successful commercial

production. The objectives of this study were to (1) determine acute tolerances for juvenile *Cherax quadricarinatus* exposed to different concentrations of ammonia, nitrite, or nitrate, and (2) estimate metabolic rates of juveniles acutely exposed to sub-lethal concentrations of ammonia, nitrite, and nitrate.

MATERIALS AND METHODS

Animals

Juvenile *Cherax quadricarinatus* were obtained from broodstock females maintained in Campbell Hall at the University of Alabama at Birmingham. Juveniles used for experiments ranged in size from 9 to 13 mm total length (10 to 25 mg wet weight) and were fed AB crayfish feed (UAB Research Foundation). This diet has previously been demonstrated to support weight gain and survival of juvenile *C. quadricarinatus* (Meade and Watts, in review). Juveniles lacking appendages (claws or walking legs) were excluded for use in experiments.

Toxicity Assays

Preliminary experiments determined the range of concentrations of ammonia, nitrite, or nitrate to be examined. For each test solution examined, 10 siblings were placed individually in polystyrene bowls containing 100 ml of the desired test solution. A minimum of two groups of siblings was used for all test solutions examined. In many cases, the variability in survival of siblings among females was high, thus numerous other groups of siblings from other females were exposed to the toxicants to increase the statistical value of mean lethal times and concentrations. All juveniles used for toxicity experiments were not fed during exposure to ammonia, nitrite, and nitrate.

Stock solutions of the toxicants were made by mixing 3.82 g reagent grade ammonium chloride, 4.92 g sodium nitrite, and 6.07 g sodium nitrate each with 1 *l* of conditioned water (28°C, pH 7.5 ± 0.2, alkalinity 70 ± 5 mg/l, hardness 300 ± 10 mg/l, and chloride 450 mg/l). Final concentrations of the stock solutions were 1000 mg/l total nitrogen in the form of NH_3-N, NO_2-N, and NO_3-N, respectively. Serial dilutions of the

stock solutions were made to attain the desired concentrations for the test solutions. Survival of juveniles was determined at concentrations of 0 (control), 25, 50, 100, and 200 mg/l total ammonia-nitrogen (NH_3-N); 0, 10, 25, 50, and 100 mg/l total nitrite-nitrogen (NO_2-N); or 0, 10, 100, and 1000 mg/l total nitrate nitrogen (NO_3-N). To determine LT_{50}s and LC_{50}s, juveniles were examined every hour for mortalities through the first 6 hr of exposure and every 6 hr thereafter for up to 120 hr (5 days). Death of the juveniles was determined by an apparent lack of movement when prodded with a blunt glass probe and by microscopic examination for a heartbeat. For all treatments, test solutions were replaced daily using static water renewal methods (Buikema et al., 1982). The dose response of juvenile crayfish was determined by plotting the probit of mortality transformed from percent mortality against log concentration (Buikema et al., 1982). Moving averages and interpolation were used to determine LC_{50} (± SD) (Buikema et al., 1982). Un-ionized NH_3-N was determined using aqueous ammonia equilibrium calculations (Emerson, 1975).

Respiratory measurments

To standardize for metabolic condition, respiratory rates of all juvenile crayfish were measured 2 days following a molt . Oxygen consumption rates were measured using a closed-chamber, temperature-controlled polarographic respirometer (Oxygraph 67097, Cyclobios-Paar, Austria) using methods described previously (Meade et al. 1994). Briefly, sibling crayfish (n = 5 / treatment) were placed in a closed respirometer and allowed to consume oxygen at tensions from fully air saturated conditions (~ 21 kPa) down to hypoxic conditions (~ 1 to 2 kPa) without the presence of ammonia or its oxidized derivatives. Juveniles were allowed to consume oxygen throughout this range of oxygen tensions in 2 trials to determine basal rates before exposure to ammonia, nitrite, or nitrate. Different concentrations of ammonia, nitrite, or nitrate were then introduced into the system and rates of oxygen consumption were again measured from air saturated to hypoxic conditions. Control crayfish were exposed to introductions of equivalent volumes

of fresh water. Juveniles were exposed to concentrations of 0, 50, 100, 250, and 500 mg/l total NH_3-N; 0, 25, 50, and 100 mg/l total NO_2-N; and 0, 100, 250, 500, and 1000 mg/l total NO_3-N. The temperature, pH, alkalinity, and hardness of the experimental solutions were similar to those conditions of the solutions used in the previous toxicity assays. Oxygen consumption rates were recorded and analyzed using Datgraf Analysis software v 2.1 (M. Reck and R. Kaufmann, Innsbruck, Austria). Oxygen consumption rates in this study are abbreviated as n_{O_2} and reported as pmol O_2/(s*mg wet weight). Statistical comparisons of oxygen consumption rates were made using the unpaired t-test (Daniel, 1987). Alpha for statistical analysis was set at 0.05.

RESULTS

Toxicity Assays

No mortalities were observed in control individuals throughout the experiments. Juvenile crayfish survived long durations when exposed to relatively moderate concentrations of ammonia (Table 1). At concentrations of total NH_3-N from 0 to 25 mg/l (0.54 mg/l total un-ionized NH_3-N), no mortalities were observed through 120 hr of exposure. At higher concentrations, survival of different groups of siblings was highly variable; average LT_{50}s for 50, 100 and 200 mg/l total NH_3-N (1.07, 2.14, and 4.28 mg/l total un-ionized NH_3-N) were 38, 19, and 13 hr, respectively. The calculated LC_{50} values for 24, 48, and 96 hr were 94.3 ± 0.24, 76.3 ± 0.12, and 45.9 ± 0.25 mg/l total NH_3-N (2.02, 1.63, and 0.98 mg/l un-ionized NH_3-N), respectively. Survival was substantially reduced when juvenile crayfish were exposed to nitrite (Table 2). At 0 and 10 mg/l total NO_2-N, no mortalities were observed through 120 hr of exposure. At higher concentrations, survival was variable among groups of siblings, but not as variable as observed for crayfish exposed to ammonia. At 25, 50, and 100 mg/l total NO_2-N, average LT_{50}s were 96, 22, and 5 hr, respectively. The calculated LC_{50} values for 24, 48, and 96 hr were 42.9 ± 0.22, 37.1 ± 0.16 and 25.9 ± 0.35 mg/ l NO_2-N, respectively. No

Table 1. Toxicity of ammonia (NH_3-N) to juvenile Australian crayfish, *Cherax quadricarinatus*.

Female #	Toxicity level Total	(Un-ionized)	LT_{50}	[a]Mean LT_{50}
I	25 mg/l NH_3-N	(0.54 mg/l)	>120 h	
II	25 mg/l NH_3-N		>120 h	
III	25 mg/l NH_3-N		>120 h	
V	25 mg/l NH_3-N		>120 h	>120h
I	50 mg/l NH_3-N	(1.07 mg/l)	48 h	
II	50 mg/l NH_3-N		48 h	
III	50 mg/l NH_3-N		24 h	
IV	50 mg/l NH_3-N		48 h	
VII	50 mg/l NH_3-N		30 h	
VIII	50 mg/l NH_3-N		24 h	
IX	50 mg/l NH_3-N		42 h	40h
I	100 mg/l NH_3-N	(2.14 mg/l)	48 h	
II	100 mg/l NH_3-N		12 h	
III	100 mg/l NH_3-N		24 h	
V	100 mg/l NH_3-N		18 h	
VI	100 mg/l NH_3-N		6 h	
X	100 mg/l NH_3-N		6 h	28h
I	200 mg/l NH_3-N	(4.28 mg/l)	24 h	
III	200 mg/l NH_3-N		12 h	
IV	200 mg/l NH_3-N		5 h	14h

[a]mean values only for siblings exposed to all levels of ammonia
note: controls (0 mg/l NH_3-N) in all experiments had no mortalities

Table 2. Toxicity of nitrite (NO_2-N) to juvenile Australian crayfish, *Cherax quadricarinatus*.

Female	Toxicity level	LT_{50}	Mean LT_{50}
I	10 mg/l NO_2-N	> 120h	
II	10 mg/l NO_2-N	> 120h	>120h
I	25 mg/l NO_2-N	96h	
II	25 mg/l NO_2-N	96h	96h
I	50 mg/l NO_2-N	24h	
II	50 mg/l NO_2-N	24h	24h
I	100 mg/l NO_2-N	6h	
II	100 mg/l NO_2-N	4h	5h

note: controls (0 mg/l NO_2-N) in all experiments had no mortalities

mortalities were observed in juvenile crayfish exposed to concentrations from 0 to 1000 mg / l total NO_3-N during the 120 hr exposure period (Table 3).

Respiratory measurements

Oxygen consumption rates varied, averaging between 3.5 and 5 pmol O_2/sec*mg wet weight, among individual juvenile *Cherax quadricatinatus* exposed to fresh water. Oxygen consumption rates were regulated over the p_{O_2} range from 18 to 4 kPa (Figs. 1, 2, and 3). Below about 4 kPa, oxygen consumption rates conformed to declining p_{O_2} until reaching zero. In most cases, once placed in the respiratory chamber, individual crayfish consumed and reduced oxygen tensions to hypoxic conditions within 30 to 45 min. When acutely exposed to ammonia at concentrations up to 1000 mg/l (total NH_3-N), no significant differences were observed in oxygen consumption rates between experimental and control individuals (Fig. 1). Observed initial oxygen consumption rates were slightly higher in individuals exposed to ammonia. Oxygen consumption rates returned to levels similar to those of control individuals after approximately 5 min. Thereafter, crayfish exposed to ammonia regulated oxygen consumption rates until conforming to declining oxygen tensions at about 4 kPa p_{O_2}.

When acutely exposed to nitrite at concentrations up to 50 mg/l (total NO_2-N), no significant differences were observed in oxygen consumption rates between experimental and control individuals (data not shown). At a concentration of 100 mg/l total NO_2-N, oxygen consumption rates in the crayfish began to decrease immediately (Fig. 2). When oxygen tensions decreased to 50% of air saturated conditions within the chamber (~ 10 kPa p_{O_2}), oxygen consumption rates of the nitrite exposed crayfish were approximately 50% of the rates of the controls ($p < 0.05$). When oxygen tensions decreased to 30% of air saturated conditions (~ 6 kPa p_{O_2}), crayfish exposed to nitrite appeared unable to consume oxygen (rates near zero).

When exposed to nitrate at concentrations up to 1000 mg/l (total NO_3-N), no significant differences were observed in oxygen consumption rates between experimental

Table 3. Toxicity of nitrate (NO_3-N) to juvenile Australian crayfish, *Cherax quadricarinatus*.

Female #	Toxicity level	LT_{50}	Mean LT_{50}
I	10 mg/l NO_3-N	> 120h	
II	10 mg/l NO_3-N	> 120h	> 120h
I	100 mg/l NO_3-N	> 120h	
II	100 mg/l NO_3-N	> 120h	> 120h
I	1000 mg/l NO_3-N	> 120h	
II	1000 mg/l NO_3-N	> 120h	> 120h

note: controls (0 mg/l NO_3-N) in all experiments had no mortalities

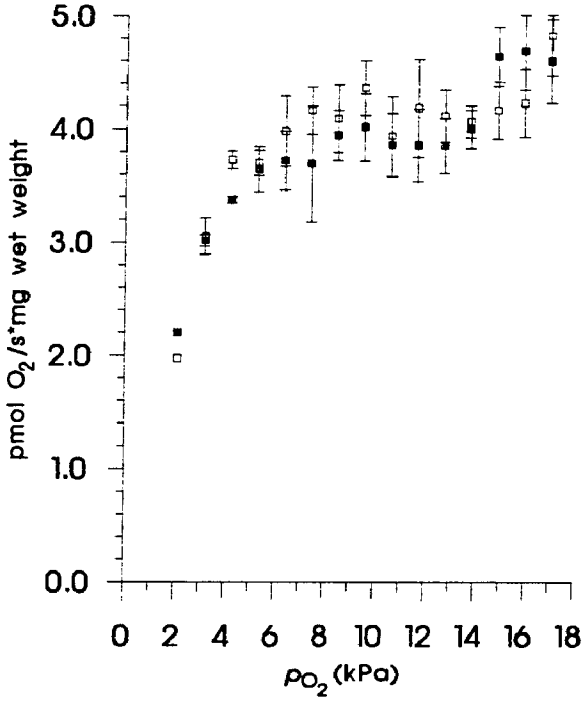

Figure 1. Oxygen consumption rates of crayfish at various p_{O_2} when exposed to fresh water (open squares) and 500 mg/l NH_3-N (closed squares).

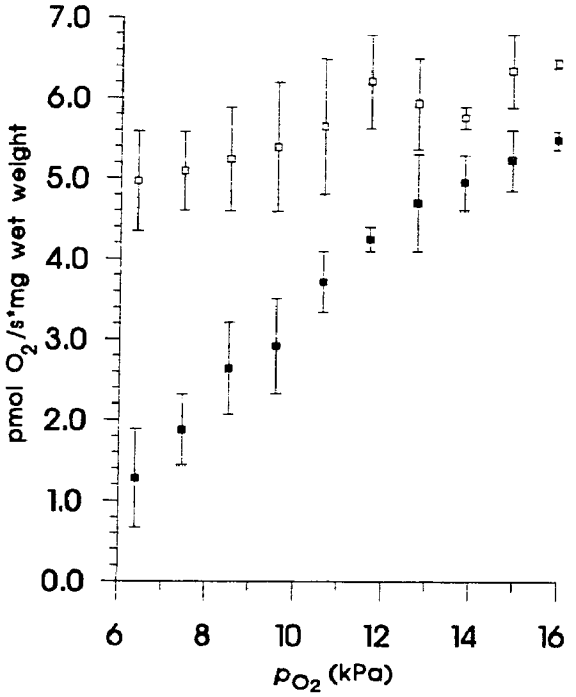

Figure 2. Oxygen consumption rates of crayfish at various p_{O_2} when exposed to fresh water (open squares) and 100 mg/l NO_2-N (closed squares).

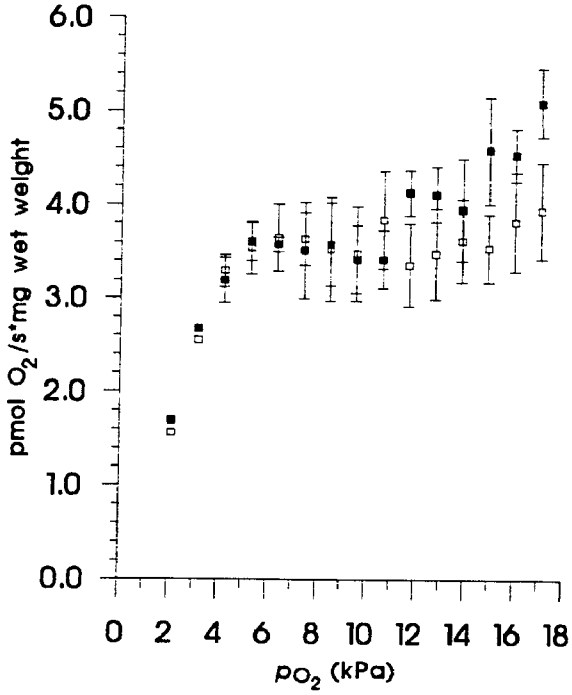

Figure 3. Oxygen consumption rates of crayfish at various p_{O_2} when exposed to fresh water (open squares) and 1000 mg/l NO_3-N (closed squares).

and control individuals (Fig. 3). As in the ammonia treatments, initial oxygen consumption rates were slightly higher than those of controls. Oxygen consumption rates also returned to levels similar to those of control individuals after approximately 5 min. Thereafter, crayfish exposed to nitrate regulated oxygen consumption rates until conforming to declining oxygen tensions at about 4 kPa p_{O_2}.

DISCUSSION

Toxicity Assays

Many fish and crustacean species exhibit similar tolerances to ammonia. For example, 96 hr LC_{50} values of approximately 1 mg/l un-ionized NH_3-N have been reported for rainbow trout (*Salmo gairdneri*), largemouth bass (*Micropterus salmoides*), and larval tiger prawn (*Penaeus monodon*) (Thurston et al., 1981a,b; Roseboom and Richey, 1977; Chin and Chen, 1987). Variability in tolerance to ammonia among the different sibling groups in this study was high and was attributed to genetic variability. Nevertheless, juvenile crayfish, *Cherax quadricarinatus*, exhibited a tolerance to ammonia similar to that observed for other crustacean species (calculated 96 hr LC_{50} values of approximately 0.98 mg/l un-ionized NH_3-N). Slightly higher ammonia tolerance, however, has been reported for other crayfish species. Evans (1979) reported 96 hr LC_{50} values between 3.2 and 3.8 mg/l total un-ionized NH_3-N for the adult crayfish, *Orconectes nais*. Liu et al.(1994) also reported 96 hr LC_{50} values of approximately 3 mg/l total un-ionized NH_3-N for juvenile (0.7 to 0.9 g) *C. quadricarinatus*. Since water conditions such as pH, temperature, dissolved oxygen, and dissolved inorganics can affect the toxicity of ammonia (for review, see Russo, 1985), differences in experimental conditions can make comparisons among toxicity studies difficult. Nevertheless, it is apparent that minimal concentrations of un-ionized ammonia can cause significant mortalities of recently-hatched juvenile *C. quadricarinatus*.

Nitrite toxicity to fish and crustaceans is highly variable from species to species. For example, 96 hr LC_{50} values of approximately 0.25, 10.25, and > 67 mg/l total NO_2-N

have been reported for rainbow trout (*Salmo gairdneri*), channel catfish (*Ictalurus punctatus*), and mottled sculpin (*Cottus bairdi*), respectively (Russo et al., 1981; Colt and Tchobanoglous, 1976; Russo and Thurston, 1977). For crayfish, 96 hr LC_{50} values of approximately 30 and 6 mg/l total NO_2-N have been reported for juvenile *Procambarus clarkii* and adult *P. simulans*, respectively (Gutzmer and Tomasso, 1985; Beitinger and Huey, 1981). Lui et al. (1994) reported 96 hr LC_{50} values for juvenile *Cherax quadricarinatus* of approximately 5 mg/l total NO_2-N at a temperature of 24 ± 1°C and a pH of 8.6 ± 0.1. In this study, 96 hr LC_{50} values of 25.9 mg/l total NO_2-N were observed for juvenile *C. quadricarinatus*. Water conditions have been observed to also affect the toxicity of nitrite, similar to the effects on ammonia toxicity (Russo, 1985), thus making comparisons among toxicity studies difficult. Chloride, in particular, at concentrations of 100 mg/l has been demonstrated to increase the resistance of crayfish to nitrite (Gutzmer and Tomasso, 1985). We suggest, however, that nitrite, in moderate concentrations, can cause significant mortalities of juvenile *C. quadricarinatus*.

Nitrate is considerably less toxic to aquatic organisms compared to ammonia and nitrite. Lethal concentrations (96 hr LC_{50}s) in excess of 500 mg/l total NO_3-N have been reported for Chinook salmon (*Oncorhynchus tshawytscha*), rainbow trout (*Salmo gairdneri*), and channel catfish (*Ictalurus punctatus*) (for review, see Russo, 1985). In this study, juvenile Australian crayfish survived long durations (>120 hr) with no mortalities when exposed to total NO_3-N concentrations of 1000 mg/l. Nitrate concentrations approaching levels of 500 mg/l total NO_3-N have been reported in large, recirculating artificial seawater systems (Pierce et al., 1993); however, concentrations in fish culture systems are seldom greater than 70 mg/l (Knepp and Arkin, 1973). Based upon the results of this study, it is unlikely that nitrate will directly affect the health of intensively cultured juvenile *Cherax quadricarinatus*.

Respiratory measurements

The mechanism of toxicity of ammonia and nitrite to fish and crustaceans is not fully understood. Several investigators have suggested that ammonia and/or nitrite may affect the health of aquatic organisms directly by influencing the oxygen binding properties of the repiratory pigments hemoglobin (Brockway, 1950; Kiese, 1974) and hemocyanin (Sanders, 1992; Needham, 1961). Indeed, Sanders et al. (1992) demonstrated that, *in vitro*, ammonia can (1) have either no effect on crustacean hemocyanin oxygen binding affinity, or (2) cause a slight increase or decrease in hemocyanin oxygen binding affinity. Either effect depends on pH and the molecular form of ammonia present. In this study, acute NH_3-N exposure, at 2.5 times the concentration which cause significant mortalities in 12 hr, did not affect the level of oxygen consumption rates or the ability of crayfish to regulate oxygen consumption rates over a wide range of environmental p_{O_2}. Although not statistically significant, slightly higher oxygen consumption rates were observed when the crayfish were inititally exposed to ammonia. These higher rates may have occurred as a result of the chemical detection of ammonia by the crayfish resulting in an excitation response. Oxygen consumption rates did, however, return to rates similar to those of control individuals, suggesting that the mechanism of toxicity of ammonia to juvenile *Cherax quadricarinatus* may not be directly associated with changes in metabolic rates. Alternatively, a change in oxygen consumption rates in juvenile crayfish may not have occurred because of the short duration (<1 hr) of exposure to ammonia in this study.

Nitrite substantially reduced the oxygen consumption rates of juvenile Australian crayfish when they were acutely exposed to concentrations that cause significant mortalities in 4 hr. In fish, nitrite binds to hemoglobin, forming methemoglobin. Methemoglobin has a reduced oxygen binding affinity when compared to hemoglobin, thus causing problems associated with oxygen transport and delivery to tissues. Needham (1961) hypothesized that nitrite can bind crustacean hemocyanin and form methemocyanins, also causing similar respiratory problems with oxygen transport and

delivery. Recent evidence suggests that nitrite can bind to crustacean hemocyanin, forming methemocyanin; however, the reaction is reversible and does not appear to affect oxygen binding properties *in vitro* (Tahon et al., 1988). Thus, the decrease in oxygen consumption rates in juvenile crayfish observed in this study may not have occurred as a result of the direct effects of nitrite on hemocyanin. Alternatively, the reduction in oxygen consumption rates may have occurred via some indirect effect on metabolism.

Nitrate, at concentrations up to 1000 mg / l total NO_3-N, did not affect the level of oxygen consumption rates or the ability of the juvenile crayfish, *Cherax quadricarinatus*, to regulate oxygen consumption rates at different environmental oxygen tensions. Oxygen consumption rates were, however, slightly elevated in crayfish when they were initially exposed to the higher concentrations and, again, may be related to the chemical detection and excitation. Nevertheless, since no mortalities were observed in juvenile crayfish after exposure to high concentrations for 120 hr, the extent of nitrate's toxic effects appears minimal in *C. quadricarinatus*.

In summary, juvenile Australian crayfish, *Cherax quadricarinatus*, demonstrate similar tolerances to ammonia, nitrite, and nitrate observed in other crustacean species. The ability of juvenile crayfish to regulate oxygen consumption rates does not appear to be affected by ammonia or nitrate during acute exposure. Nitrite, however, inhibits the ability of juvenile crayfish to regulate oxygen consumption rates. At low environmental p_{O_2}, this effect can substantually impair respiratory functions and affect the potential survival of juvenile crayfish. Further studies are needed to examine the long term effects of sublethal exposure to ammonia and nitrite on the oxygen consumption rates of this juvenile crayfish.

ACKNOWLEDGMENTS

We thank Dr. Jeanette Doeller and Dr. David Kraus for technical assistance in the respiratory measurements. This research was supported in part by the Alabama Academy of Sciences to MEM and by NSF IBN9118048 to JED and DWK.

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SUMMARY

Diet substantially affects weight gain and survival of juvenile Australian crayfish, *Cherax quadricarinatus*. Growth patterns, both molt intervals and molt increments, vary with diets that promote differential weight gain. Currently, commercial crustacean feeds do not provide for the nutritional needs of this crayfish species. Preliminary use of diets formulated specifically for crayfish suggests that improved feeds may improve commercial production. The assumed large range of physiological tolerance of *C. quadricarinatus* suggests that culture of this species could be accomplished in a variety of conditions. *C. quadricarinatus* has the ability to tolerate low environmental oxygen tensions. In situations where this species is stocked at high densities, culturists do not have to worry about short term problems associated with low oxygen tensions; however, the species will not tolerate anoxic environments. *C. quadricarinatus* also tolerates a wide range of environmental temperatures. However, the range of temperatures where maximum growth and survival occurs is rather narrow (24-28°C). *C. quadricarinatus* has a very low tolerance to saline conditions. In areas where freshwater resources are not readily available, short term maintenance of individuals in saline water is possible; however, long term culture in saline water is not recommended. Finally, *C. quadricarinatus* tolerates levels of nitrogenous compounds (ammonia, nitrite, and nitrate) similar to those reported to be tolerated by other crayfish and crustacean species. Overall, *C. quadricarinatus* is a hearty crayfish species that, if properly managed, could be considered for intensive commercial culture. Further evaluation of nutrient requirements and the subsequent development of practical diets which elicit maximal growth and survival is needed.

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GRADUATE SCHOOL
UNIVERSITY OF ALABAMA AT BIRMINGHAM
DISSERTATION APPROVAL FORM

Name of Candidate Mark E. Meade

Major Subject Biology

Title of Dissertation Effects of diet and environmental factors on growth, survival, and physiology of juvenile Australian crayfish, Cherax quadricarinatus

Dissertation Committee:

Stephen J. Watts, Chairman

David W. Kraus D.S.

James McClintock D.S.

Louis R. D'Abramo D.S.

David Rouse D.S.

Director of Graduate Program Daniel D. Jones

Dean, UAB Graduate School John F. Fisher

Date 4/19/95