Title: THE EFFECTS OF WATER VELOCITY, PHOTOPERIOD AND SUBSTRATE ON THE SURVIVAL AND GROWTH OF SUBYEARLING OREGON CRAYFISH (Pacifastacus trowbridgii) STIMPSON

Abstract approved: John R. Donaldson

In an effort to enhance the possibilities of commercially rearing crayfish (Pacifastacus trowbridgii), the subyearling segment of the life cycle was examined to determine practical ways of increasing survival and growth. Four experiments were designed to study the effects of water velocity, photoperiod, substrate and density of stocking on the survival, growth and habitat preference of subyearling crayfish. Water velocities ranging from 4 cm/sec to 10 cm/sec were found to have a positive effect on both survival and growth. Survival was greatest under light-dark conditions, while the maximum growth was attained in total darkness. The optimal density of stocking was found to be between 50 and 100 subyearlings per m². A habitat consisting of 3-8 cm gravel and alder leaves was found to be a preferred habitat.
The Effects of Water Velocity, Photoperiod and Substrate on the Survival and Growth of Subyearling Oregon Crayfish (*Pacifastacus trowbridgii*) Stimpson

by

Mark Irvin Hutton

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APPROVED:

Redacted for Privacy

Associate Professor of Fisheries in charge of major

Redacted for Privacy

Head of Department of Fisheries and Wildlife

Redacted for Privacy

Dean of Graduate School

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Typed by Opal Grossnicklaus for Mark I. Hutton
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THE EFFECTS OF WATER VELOCITY, PHOTOPERIOD AND SUBSTRATE ON THE SURVIVAL AND GROWTH OF SUBYEARLING OREGON CRAYFISH (Pacifastacus trowbridgii) STIMPSON

INTRODUCTION

The natural stocks of crayfish in the Pacific Northwest have been over-exploited and, at present, the local demands and those from Sweden and Europe are much greater than the apparent supply. Attention must be turned to the culturing of crayfish to meet these demands. It is the overall objective of this project to examine certain critical stages in the life history and biology of the crayfish, Pacifastacus trowbridgii, which would be essential to a successful culture program.

Life History

The current literature has been thoroughly reviewed recently by Coykendall (1973), so I will review only new and pertinent information. The life history of P. trowbridgii has been carefully recorded by Mason (1963) and Miller (1960) and reviewed by Coykendall (1973). The general reproductive activities have been described by Mason (1963), Johnson (1971) and Abrahamsson (1971). Females mature as early as two years and undergo rapid ovarian development in September and October, as water temperatures decline. Large polygamous males mate with the females during which sperm packets are transferred externally. An excellent account of the copulation of
P. trowbridgii is given by Mason (1970b). The ovum are fertilized on the female as they are extruded from the genital pore at the base of the third pair of walking legs. The zygote is then attached to the ventral side of the abdomen and undergoes development for a period of five to six months (Miller, 1960; Mason, 1970a; Abrahamsson, 1971; Johnson, 1971 and Coykendall, 1973).

After hatching, Stage I crayfish remain attached to the female setae and are completely dependent on the female. In approximately one week the Stage I crayfish molt into Stage II crayfish and are totally independent of the female. At this stage, they must begin foraging for their food and providing for their own protection. This becomes a critical period in their life history as Stage II crayfish are vulnerable both to inter- and especially intra-specific predation.

The feeding habits, molting frequencies and habitats of Stage II crayfish have been well documented. Mason (1963) has shown their diet to consist of 65% animal matter and 35% plant material. This high protein diet enhances the early growth rate, as evidenced by the frequency of molting found by some experimenters. Chidester (1912) reported that young crayfish molt frequently during the first year. Mason (1963) recorded an average of 12 molts during the first year. Coykendall (1973) observed a single subyearling to molt four times in a 45 day period. Researchers have recorded the habitat in which young crayfish were found or in which they had good success.
in rearing them. Shallow flowing water with abundant cover and vegetation was observed to be the preferred habitat of subyearling crayfish by Andrews (1907) and Tack (1939).

Many researchers have reported low survival for subyearling crayfish. Andrews (1904), using *Cambarus affinus*, observed only eight crayfish (from the hatch of one female), to survive four and one-half months in a fresh water aquarium. Paladino (1966) reported that losses were great during the first few months of life for the crayfish *Astacus astacus*, *A. leptodactylus* and *Orconectes limosus*. Paladino cited cannibalism as a major reason for this extremely high mortality. Bovbjerg (1956) felt the survival value of this aggressive behavior accounted for the low densities of crayfish found in nature.

Post-yearling or adult crayfish change significantly in their food consumption, habitat and growth (Mason, 1963) from the habits previously described for subyearling crayfish. Mason (1963) and Chidester (1912) have demonstrated that adult crayfish are opportunistic vegetarians and inhabit deeper water than the subyearlings.

Molting frequencies have been reported to decrease to six molts in the second year, three molts in the third year, two molts in the fourth year and one molt a year for up to eight years (Mason, 1963). Mature males and females are capable of mating every year, but seldom mate over two or three times.
Culturing Problems

There are several critical phases in the culturing of *Pacifastacus* spp., that need to be understood in order to successfully rear crayfish for profit. These areas are: (1) brood stock, (2) hatching, (3) post-hatching, (4) post-yearling and (5) nutrition.

Brood Stock

A controlled brood stock is essential for any culture program. There has been a great deal of work done with various aspects of adult maturation. Stephens (1951) found that manipulation of the photoperiod induced changes in the ovarian cycle in *Cambarus* spp. Aiken (1969) found that ovarian maturation and egg laying in *Orconectes virilis* could be controlled by temperature and photoperiod. Brodskii (1963) suggested the need for large adult spawning farms for crayfish. Perryman (1969) reported that the reproductive cycle in *Procambarus* spp. could be regulated by changing the photoperiod. Westman (1970) has successfully reared and maintained a brood stock of *Pacifastacus leniusculus* in Finland. Zukerzis (1971) has regulated a brood stock in Lithuanian hatcheries. Coykendall (1973) found that spawning could be induced in the laboratory earlier than spawning in nature by manipulating the temperature.
Hatching

The development of hatching techniques must follow control of the brood stock. Hatching time must be reduced and controlled to allow mass egg hatching, in space saving designs, with minimum mortality. Recently this problem has received some attention. As early as 1906, Andrews (1907) demonstrated that Astacus (Pacificastacus) could be hatched and reared in the laboratory. Tsukeris (1962) successfully incubated Astacus astacus eggs in fish hatching facilities. Brodskii (1963) foresaw the need to establish multipurpose incubation plants for crayfish eggs. Westman (1970) and Zukerzis (1971) have been successful in hatching Pacifastacus leniusculus. Mason (as reported by Coykendall, 1973) was able to successfully hatch young P. trowbridgii in unheated Heath incubation trays, by removing the eggs from the abdomen. Perkins (1972) has studied the effects of temperature on the growth and development of the closely related lobster (Homarus americanus) which may add to the technology necessary for the acceleration of hatching time.

Post-hatching

Post-hatching care of crayfish is important to culture programs, as the losses incurred during this time can be as great as 90%. It is known that crayfish are extremely cannabalistic on other molting
crayfish (Bovbjerg, 1956; Mason, 1963; Momot, 1967; Paladino, 1966 and Coykendall, 1973). Westman (1970) and Zukerzis (1971) have reported solving at least some of these problems in their successful crayfish hatcheries, but did not furnish any details. Because the post-hatching problems with *P. trowbridgii* may be different it is necessary to focus special attention to this particular crayfish. Coykendall and I have postulated that the surviving crayfish are the slowest growing crayfish, as the fastest growing crayfish molt first and are cannibalized by other crayfish who have not molted or who are slower growing. This presents a problem to the potential crayfish culturist.

**Post-yearling**

The rearing of post-yearling crayfish in an intense culture situation, is always difficult. Crayfish must be maintained under high densities with a maximum of growth and a minimum mortality. Rearing has always been accomplished in a large pond complex. Great success is enjoyed in Louisiana with this type of culture. Ham (1971), Avault *et al.* (1970) and Lacaze (1960) report that the southern crayfish culture is uncomplicated and consists of shallow rearing ponds, with vegetation, irregular stocking rates and periodic harvesting of the adults. Techniques developed to culture the Louisiana crayfish *Procambarus clarkii* and *P. clarkii ocutus* do not appear
to be applicable to the Pacific Northwest species, based on differing life histories and biology. To my knowledge, no one in the Northwest is successfully pond rearing *Pacifastacus* spp. It is undetermined at this time if pond culture would be suitable for the commercial rearing of *Pacifastacus* spp.

**Nutrition**

The fifth major area for consideration is the nutritional requirements for crayfish. Utilizing exact nutritional requirements for crayfish would maximize growth and minimize cost of feeding, both essential components in a successful culture program. Stomach analysis by Mason (1963) and food observations by Miller (1960) confirm that subyearling crayfish consume more animal matter than plant material. The trend is reversed in post-yearling crayfish, as their diet shifts to almost 90% plant material. It is not known whether this shift is caused by food preference, food availability or dietary needs. Coykendall (1973) examined two diets for subyearling and yearling crayfish and found fresh frozen marine euphasiids to be preferred over rabbit pellets. Some information may be derived from studies by Meyers *et al.* (1972), who have studied crustacean diets and dietary needs.
Background Information

It is apparent from the literature, or lack of it, that the majority of all crayfish studies have not been concerned with the culturing of *Pacifastacus* spp., which creates the need to investigate this genera. After observing crayfish in the field and in the laboratory, and reviewing the sparse information available, the principal objective became the investigation of post-hatching problems.

**Flowing Water and Substrates**

Mason (1963) reported seining subyearling crayfish in the shallow riffles or in other shallow water areas in Berry Creek, Oregon. When collecting specimens for these experiments I observed subyearling crayfish mostly in shallow, flowing water, in and around coarse gravel and leaf packs. Because of these observations, and the accompanying void in the literature concerning flowing water designs, flowing water will be compared to standing water survival and growth of the subyearling crayfish. Substrate preference will also be tested.

**Mortality**

In the literature are cited many examples of mortality on subyearling crayfish; Andrews (1904), Bovbjerg (1956), Paladino (1966)
and Coykendall (1973). A distinct clumping action appears to be a major reason for this high laboratory mortality in standing water. Mason has observed Stage II _Pacifastacus trowbridgii_ in tight clumps or clusters for up to ten days. My observations verify what Mason found. Every spring after hatching, this clumping action occurs and I have observed 95-99% mortality over a few weeks time in fresh water tanks.

The commercial rearing of crayfish would require high densities of crayfish to be reared in order to be economical. Therefore mortality due to clumping would be a potential problem that would need solving.

**Photoperiod**

It is commonly accepted that crayfish, including _Pacifastacus_ spp. are nocturnal (Mason, 1963). Mason (1970c) found _P. trowbridgii_ subyearling were negatively phototrophic. Roberts (1944) reported that _Cambarus virilis_, when exposed to light, released a chemical from the eyestalk that caused locomotor tendencies to become greatly reduced. Roberts also found that the metabolic rate was greatly reduced in light or during the day. If daylight feeding involved searching for the food, locomotion and vision would be required and the chances of increased growth or increased food consumption would be better when the locomotor tendencies and eyestalk
operation for vision are not inhibited.

Subyearling crayfish will therefore be subjected to a normal day length photoperiod and to a completely dark period; testing both growth and survival.

**Objectives**

The specific objectives of this research are the investigation of the roles that water velocity, photoperiod and substrate play in the growth, survival and distribution of subyearling crayfish (*Pacifastacus trowbridgii*). This investigation is built, in part, on earlier experiments by Coykendall (1973). It is one phase of a long term crustacean culture research program, which would benefit not only potential crayfish culturists but also other crustacean culture schemes.
MATERIALS AND METHODS

Experimental Design

It was necessary to design four separate experiments to meet the objectives set forth in the introduction as each experiment was different but yet overlapping in some way with another experiment. Experiment I was designed to study velocity preference and survival of subyearling crayfish. Experiment II was designed to study velocity preference, survival and growth of subyearling crayfish. Experiment III was designed to study the habitat preference of subyearling crayfish. Experiment IV was designed to study subyearling survival and growth in the preferred habitats and water velocities.

Materials

The experiments were conducted at the Oak Creek Fisheries Laboratory, Department of Fisheries and Wildlife, Oregon State University, Corvallis, in Artificial Stream Laboratory Number One.

Stream Design

The lab contained six artificial streams, each equal in size, capacity and design. False stream bottoms were constructed of PVC window screening to allow a flow of water to pass underneath to aid in eliminating anaerobic conditions that could form in the sand, leaves and rocks making up substrate. PVC window
screening was also used to form end screens for the experimental areas in the streams to exclude test crayfish from areas of the paddle wheels, drain pipe, oyster filters and end areas. A complete description of the basic stream design is found in McIntire et al. (1964).

Figure 1 illustrates the experimental stream arrangement, showing paddle wheel location and photoperiod design. The Roman numerals depict the sections of stream in which Experiments I, II, III and IV were conducted.

Paddle Wheel and Motor Arrangement

Four of the six streams were equipped with paddle wheels to provide current for the streams. The paddle wheels were driven with a one-quarter horse motor, using a right-angle worm gear reducer and a common drive shaft. The approximate speed of the paddle wheels was regulated by the size of the pulley wheels, while the finer adjustments were made by variable speed pulleys attached between each paddle wheel and the common drive shaft. At times it was necessary to rearrange the rocks at the head of each stream to make the final adjustments in stream velocity.

Light Regulation

The lab was poorly insulated and poorly designed for any type
Figure 1. Diagram of experimental tank arrangement, water flow and paddle wheel operation. Roman numerals indicate section of stream utilized in Experiments I, II, III and IV.
of photoperiod control. Each window was therefore covered with heavy black plastic to seal out light and act as an absorbant of sunlight to aid in warming the building. Three streams were enclosed within hanging black plastic curtains to keep out the light while the other three were equipped with hanging florescent lights regulated by an electric timer.

**General Methods**

There were several methods of procedure common to all experiments. To avoid endless repetition these procedures will be presented first, while the individual methods that differ with each experiment will be presented later.

**Collections**

Crayfish used in this study were collected from five streams; all located in Benton County, Oregon. Buried females and subyearlings were collected in three streams: Rock Creek, five miles west of Philomath; Big Elk Creek, three miles west of Harlan and North Fork Alsea, one-half mile above the Alsea Fish Hatchery. Subyearlings were collected in two streams: Lobster Creek, one mile west of Lobster Valley and Luckiamute River, five and one-half miles west of the Oregon Highway 223 junction with Hoskins Road.
Stocking

Stocking procedures were always the same; the total number of crayfish to be used were randomly distributed equidistant throughout the stream. The experiment was started the instant the crayfish were stocked. I found this to be necessary for two reasons; (1) several crayfish were observed to travel the entire length of the stream from the fast velocity to the slowest velocity in less than 20 minutes and (2) individual mortalities could not be recorded once the crayfish were stocked so an accurate assessment of survival necessitated that the experiment begin at the time of stocking.

Temperature Control

Water was supplied from a gravity flow system, into a 12 cubic foot head box, where it was heated to 20°C with a 2000 Watt heating coil and distributed to each of six tanks at a rate of one liter per minute per tank. The temperature was monitored daily with a hand thermometer.

Feeding

The original diet consisted of equal portions of fresh alder leaves, tubificid worms and fresh frozen marine euphasiids. Crayfish were fed every other day as it was found that there was always
food remaining in the stream at the time of the next feeding. An equal amount of each food was placed at the upstream end of each square foot section of stream to compensate for the down stream drift of food with the current. The amount placed in each square foot section was always more than the entire experimental population of crayfish could eat. This eliminated food availability as a major factor influencing stream dispersion. After 12 days of the first experiment it was discovered that there was a fouling problem with the marine euphasiids.

Coykendall (1973) used marine euphasiids and reported no fouling problem mainly because he cleaned all surplus food daily with the tanks. However, in my experiments, the euphasiids putrefied if left in the water for two days and because of the nature of the stream bottom and the uncertainty associated with cleaning in the dark tanks it was decided to eliminate the marine euphasiids from the remainder of the first experiment and from the last three experiments.

**Photoperiod**

A normal day length photoperiod was compared to a completely dark period in all experiments. The photoperiod was designed to duplicate the actual day light period for that particular time of year when the crayfish were collected. These experiments began during
the summer solstice. Since day length decreased for each succeeding experiment the light-dark cycle was left untouched to provide constant light source for all comparisons between experiments. The artificial light illumination was 84 foot-candles.

Aeration and Filtration

Oxygen was added to the two no-velocity tanks for Experiments II, III and IV for the following reasons: (1) the mortality observed in Experiment I was unusually high, (2) putrified euphasiids and heavy growths of algae and bacteria were creating a high demand for the available oxygen, (3) water was scummy in appearance and (4) current literature stressed the importance of near saturation oxygen levels for the care of young crayfish (Paladino, 1966; Coykendall, 1973 and Moshiri et al., 1971).

Each stream contained a small supply of oyster shells, placed near the paddle wheels to help filter crayfish nitrogenous wastes from the water.

Termination

All experiments were terminated using the same procedure: (1) water was drained from the tank, (2) crayfish were removed and the section of the stream in which they were found was recorded and (3) border line crayfish were recorded in the upstream section.
Water Velocity

Water velocity was created by the paddle wheels. The PVC screens forming the stream bottoms were sloped to provide an increasing stream depth thereby effecting a decreasing water velocity down the trough. Depth of the water ranged from 3 cm at the shallow end to 10 cm at the deep end. Water velocity was measured by a Nypric micro-current meter, to the nearest square centimeter per second (cm/sec). Four velocity measurements were taken in each square foot of stream and the average of the four was used. Velocity measurements were made at the beginning and end of each experiment with no appreciable differences detected.

Measurements

Growth was measured using three methods: (1) wet weight comparisons, (2) carapace length comparisons and (3) dry weight comparisons. It is usual to use at least two measurements to assess growth in crustaceans. Growth is difficult to assess using only wet weight measurements, because the percentage of water in the animal varies greatly according to the time of molting. The use of two or three measurements of the same animal allows for a more accurate measurement of growth.

Wet weights were measured to the nearest 0.01 gram. Crayfish
to be weighed were placed in a box lined with paper towels for two minutes, then in a smaller box lined with Kimwipes for one minute. Each crayfish was then blotted with a dry Kimwipe to remove any access water. They were placed one at a time in a beaker of water tared to zero and weighed. After the weighing of each crayfish the beaker was re-tared to zero before adding the next animal. At the end of each experiment the drying procedure was exactly the same, however crayfish were weighed out of water in aluminum weighing trays.

Carapace lengths were recorded from the posterior orbit of the eye to the dorsal-mid posterior edge of the carapace. A lighted magnifying lens was used to aid in each measurement. All measurements were taken with a Fowler Dial caliper to the nearest 0.1 mm. Measurements were taken immediately after the crayfish had been blotted for the last time, just prior to weighing.

Dry weights were used to supplement growth information for Experiment IV. The crayfish were placed in a drying oven at 65°C for 96 hours and then in a desiccator for 24 hours and weighed to the nearest 10⁻⁴ gram.

Growth

Growth was calculated as the mean average relative growth rate expressed in terms of percent increase per day. The equation
is from Warren (1971) and is as follows:

\[
\text{Daily Average Relative Growth Rate} = \frac{X_2 - X_1}{0.5 (X_1 + X_2)(\text{time})} \quad (100)
\]

where

\[
X_1 = \text{initial measurement (weight or length)}, \\
X_2 = \text{final measurement (weight or length)}, \\
\text{time} = \text{duration of experiment in days}.
\]

Because the experimental design required comparisons of initial and final means the equation was modified and is as follows:

\[
\text{Mean Daily Average Relative Growth Rate} = \frac{\bar{X}_2 - \bar{X}_1}{0.5 (\bar{X}_1 + \bar{X}_2)(\text{time})} \quad (100)
\]

where

\[
\bar{X}_1 = \text{mean initial measurement (weight or length)}, \\
\bar{X}_2 = \text{mean final measurement (weight or length)}.
\]

This use of the equation does not utilize the means of individual growth rates but rather the growth rate represents the mean measurements from the initial and final groups of crayfish.

**Yield**

Yield was calculated as the total gain in biomass and is given in the following formula.
Yield = \frac{n_f(\bar{w}_f) - n_i(\bar{w}_i)}{m^2 \text{ Time (days)}}

where

\begin{align*}
n_i \text{ and } f &= \text{number of crayfish - initial and final} \\
\bar{w}_i \text{ and } f &= \text{mean wet weight - initial and final in grams} \\
m^2 &= \text{area in meters squared}
\end{align*}

**Individual Experimental Procedures**

**Experiment I**

Experiment I was designed to test the influence of three ranges of water velocities (0 cm per second, 5-7 cm per second and 7-10 cm per second) and two photoperiods on the survival and velocity preference of subyearling crayfish. The experiment was designed to run 30 days (June 4 to July 5).

Test animals came from three sources: (1) Rock Creek berried females held through the winter, (2) Alsea River females spawned in the lab and held through the winter and (3) Elk Creek females gathered in the spring carrying Stage I and II juveniles. All females were placed in warm water to induce the young to come off together. After the Stage II's had dropped from the females, there was a large mortality, as the young clumped together in the bottom of the aquaria.
They were then moved to a holding tank with colder water where they were left until enough could be gathered to begin the experiment. The colder water worked to reduce their metabolic rate and slow down aggression. The total time spent in the cold water holding tank ranged from five days for some crayfish to ten days for others.

They were stocked in the tanks at a rate of 120 per stream. Water temperatures in the streams were identical to the water they had been held in. They were acclimated to 20°C by raising the temperature 1°C each day until the temperature was 20°C. After this initial rise in temperature there was an unexplainably large fluctuation in the daily maximum and minimum temperatures. On the 24th day it was accidentally discovered that the water heater was connected into the same circuit as the day-night timer, which caused the heater to be turned off at night and on only during the day. The mistake was corrected and no more problems were incurred.

The feeding design was altered after only 10 days of the experiment. Surplus food was not cleaned out of the tanks, therefore a fouling problem developed with the marine euphasiids. When temperatures reached near 20°C the euphasiids would generate bacterial growth and the putrification would turn the water sour. This was especially evident in the standing water streams. The euphasiids were immediately eliminated from the diet leaving only the alder leaves and the tubificid worms.
Experiment II

Experiment II was designed to add strength to the results of the first experiment. It was modified to add a growth comparison to the survival and velocity preference already described.

Subyearling crayfish for this experiment were collected from Rock Creek with a hand seine. They were acclimated to 20°C for a period of seven days after which they were stocked at a rate of 30 per stream. The crayfish were starved 48 hours prior to the beginning of the experiment. Initial wet weights and carapace lengths were taken and recorded as described earlier. They were fed to repletion on tubifex worms and alder leaves. The experiment lasted 17 days (July 15 to July 30); after which final wet weights and lengths were taken.

Experiment III

Experiment III was designed to establish habitat preference. Four habitats were picked: (1) three to eight cm gravel, (2) sub three-cm gravel, (3) sand and (4) alder leaf packs. The two sizes of gravel were picked to demonstrate a size preference in habitat, if any. Sand was used to provide an easy place to obtain food and also to be used to fulfill the requirement of the statocyst organs. The alder leaf packs were used as they represented a source of food.
and a likely habitat. Experimental animals came from Big Elk Creek and Lobster Creek.

Each substrate occupied a 387 square centimeter portion of the stream section; the total area used in the experiment being 1.155 meters squared (Figure 1). Because of trends already displayed for certain water velocities, the lowest velocity in each tank was used; that being 7 cm per second in both fast velocity tanks and 4 cm per second in the two slow velocity streams.

Placement for each of the four habitats was random. The experiment was repeated four times so that habitat preference was based entirely on the substrate present and not on position in the stream. Figure 2 illustrates the experimental design.

Water was not heated and fluctuated between 14 and 16°C. Crayfish were fed twice during each experiment; on the first and third days. At the end of the experiment food was always present in each section so food availability was not responsible for habitat selection. Termination procedures were as described in previous sections except (1) habitats were distinct in boundaries so there were no borderline cases and (2) termination was always between 10:00 and 12:00 a.m. to eliminate diel distribution tendencies.

Experiment IV

Experiment IV was designed to combine the preferred habitats from Experiment III with the preferred velocities of Experiments I and II and to measure growth comparing these conditions with the
Figure 2. Habitat placement for the four sub-experiments in Experiment III. Each arrangement was tested in all six streams.
different photoperiods. Growth was measured using the previously
described methods for wet weights and carapace lengths and also with
dry weights. This was accomplished by establishing a wet weight to
dry weight correlation and linear regression with an initial subsample
equal to half the experimental population. From 180 crayfish, 60
were used in the subsample and 120 were used in the experiment.

Using the regression line: \( Y = -.00128 + .28282 \times \), from the
subsample, experimentally measured initial wet weights were con-
verted to calculated initial dry weights; with a regression coefficient
of .939.

The formula used for the expression of dry weight growth is
another modification of the basic equation by Warren (1971):

\[
\text{Mean Daily Average Relative Growth Rate} = \frac{\overline{X}_2 - \overline{X}_1}{0.5 (\overline{X}_1 + \overline{X}_2) (\text{time})} \tag{100}
\]

where

\[ \overline{X}_1 = \text{mean calculated initial dry weight}, \]
\[ \overline{X}_2 = \text{mean measured final dry weight}. \]

All animals were obtained from the Luckiamute River with a
hand seine. They were sorted for size such that the animals used
were the 180 closest in length. Acclimation took place in the holding
box prior to the experiment for six days. They were starved 48 hours
prior to the initial weighing and the beginning of the experiment.
They were fed repletion diet throughout the entire experiment, after which final wet and dry weights and lengths were recorded. The experiment ran for 24 days (September 7 to October 2).
RESULTS

Results from Experiments I through IV will be compared in terms of velocity preference, survival, growth and yield. A presentation of results by Experiment would not only be confusing but unrelated and difficult to discuss.

Velocity Preference

The preference of subyearling crayfish for specific water velocities relative to different photoperiods and densities is shown in Figure 3. There appears to be little effect due to the different stocking densities on velocity preference. However, there was a definite trend established for crayfish in the slow and fast velocity tanks to migrate to the section of lowest velocity.

Velocity preference for pooled densities and pooled photoperiod treatments is shown in Figure 4. These data graphically depict the trend of migration to slower moving water. Figure 4 also represents velocity preference as it may have been affected by stream depth. As was mentioned earlier, the stream bottoms were uniformly increased in depth to provide the decreasing stream velocity essential to the experiments. With reference to the no-velocity tanks in Figures 3 and 4, there is no apparent depth preference to bias the results of the velocity preference for the subyearling crayfish.
Figure 3. The influence of stream velocity and photoperiod on the distribution of crayfish at two stocking densities for Experiments I and II.
Figure 4. The influence of velocity on the combined results of photoperiod and densities of subyearling crayfish in Experiments I and II.
Survival

The comparisons of survival between different densities of crayfish, both photoperiods and all water velocities are found in Figure 5. Different stocking densities resulted in the need to graph not only the percent survival but the actual number of surviving crayfish in order that a correct assessment of the results might be made. Survival in the no-velocity tanks of Experiment I was much lower than survival in the slow-velocity tanks in the same experiment. No obvious conclusions can be drawn from Experiment II except that:

1) there was only slightly greater survival in the light-dark tanks and
2) there was a slightly greater survival in the fast velocity tanks.

Survival for Experiment IV is presented in Figure 6. There was no obvious difference due to velocity or photoperiod in the survival rates for Experiment IV. Survival in the fast-velocity tanks was only slightly greater than survival in all other tanks.

Habitat Preference

Water velocity and photoperiod had no effect on habitat preference for subyearling crayfish. Habitat preference for combined water velocity and photoperiod treatments are presented in Figure 7. There was an almost equal and unanimous preference for 3-8 cm gravel and the alder leaf pack. Very few individuals were found in
Figure 5. Crayfish survival in numbers and percent for Experiments I and II.
Numbers of crayfish

<table>
<thead>
<tr>
<th></th>
<th>No velocity</th>
<th>Slow velocity</th>
<th>Fast velocity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light-Dark</td>
<td>10</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Light-Dark</td>
<td>100</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Light-Dark</td>
<td>100</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

Figure 6. Percent survival for subyearling crayfish in Experiment IV.
Figure 7. A comparison of subyearling crayfish habitat preference combining photoperiod, velocities and sub-experiments.
the sand or in the sub-3 cm gravel.

**Growth**

Means of group wet and dry weights and carapace lengths were used to assess the mean daily average relative growth rate for the various treatments and experiments. The use of maximum and minimum growth parameters are not possible in this situation because mean group measurements were used to record growth information and not mean growth rates.

Statistical analyses of growth results were not possible. Such analyses depend on there being no significant differences in the size of the crayfish at the beginning of the experiment. A one-factor analysis of variance was used to determine if there were differences. There were significant differences at the 99% confidence level, therefore no statistical tests could be performed on the final measurements or on the mean relative growth rates. All analyses must come from strict interpretation of the results in the graphs or tables.

Water velocity and photoperiod have an effect on the growth of the subyearlings as illustrated in Figures 8 and 9 and presented in Tables 1 and 2, for Experiments II and IV respectively. In both experiments, growth measured in completely dark tanks, for both the no-velocity and slow-velocity tanks was much greater than the growth measured in the normal day length photoperiod. However,
Figure 8. Relative growth rates for carapace lengths and wet weights for subyearling crayfish in Experiment II.
Figure 9. Relative growth rates for carapace lengths and wet weights for subyearling crayfish in Experiment IV (24 days).
Table 1. The effects of water velocity and photoperiod on the growth rates of subyearling crayfish: Experiment II.

<table>
<thead>
<tr>
<th>Water velocity</th>
<th>Photoperiod</th>
<th>Growth Rate: Length (% per day)</th>
<th>Growth Rate: Wet Weight (% per day)</th>
<th>Survival %</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Light-dark</td>
<td>1.384</td>
<td>4.339</td>
<td>60</td>
</tr>
<tr>
<td>None</td>
<td>Dark</td>
<td>1.772</td>
<td>4.793</td>
<td>70</td>
</tr>
<tr>
<td>Slow</td>
<td>Light-dark</td>
<td>1.247</td>
<td>4.234</td>
<td>70</td>
</tr>
<tr>
<td>Slow</td>
<td>Dark</td>
<td>1.823</td>
<td>4.402</td>
<td>63</td>
</tr>
<tr>
<td>Fast</td>
<td>Light-dark</td>
<td>1.832</td>
<td>4.712</td>
<td>90</td>
</tr>
<tr>
<td>Fast</td>
<td>Dark</td>
<td>1.843</td>
<td>4.609</td>
<td>80</td>
</tr>
</tbody>
</table>

Table 2. The effects of water velocity and photoperiod on the growth rates of subyearling crayfish: Experiment IV.

<table>
<thead>
<tr>
<th>Water velocity</th>
<th>Photoperiod</th>
<th>Growth Rate: Length (% per day)</th>
<th>Growth Rate: Wet Weight (% per day)</th>
<th>Growth Rate: Dry Weight (% per day)</th>
<th>Survival %</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Light-dark</td>
<td>0.735</td>
<td>2.083</td>
<td>1.576</td>
<td>45</td>
</tr>
<tr>
<td>None</td>
<td>Dark</td>
<td>1.311</td>
<td>3.659</td>
<td>2.852</td>
<td>45</td>
</tr>
<tr>
<td>Slow</td>
<td>Light-dark</td>
<td>1.033</td>
<td>3.153</td>
<td>2.996</td>
<td>45</td>
</tr>
<tr>
<td>Slow</td>
<td>Dark</td>
<td>1.388</td>
<td>3.986</td>
<td>3.621</td>
<td>45</td>
</tr>
<tr>
<td>Fast</td>
<td>Light-dark</td>
<td>1.429</td>
<td>3.873</td>
<td>3.633</td>
<td>55</td>
</tr>
<tr>
<td>Fast</td>
<td>Dark</td>
<td>1.373</td>
<td>3.604</td>
<td>3.084</td>
<td>50</td>
</tr>
</tbody>
</table>
in the fast-velocity tanks the light-dark photoperiod represented better growth in both experiments. Graphically there appeared to be little or no difference in growth due to velocity. The only clear picture was that growth was always less in the no-velocity tanks and greater in slow- and fast-velocity tanks for carapace length comparisons. The difference in growth due to velocity does not appear to be constant between experiments for wet weight growth comparisons.

Relative growth rates using dry weights from Experiment IV are found in Figure 10. A very prominent pattern shows higher growth rates in both the slow- and fast-velocity tanks, than in the no-velocity tanks. The trend established for no-velocity and slow-velocity wet weights and carapace lengths in the dark tanks is again greater than the growth rate found in the light-dark tanks. The exception once again is the fast-velocity tank, where the light-dark growth rate is greater than the dark tank growth rates (Table 2).

Yield

A comparison of the total yield for each tank representing all velocities and photoperiods for Experiments II and IV may be found in Figures 11 and 12 and in Table 3. Subyearling crayfish held in flowing water had greater growth and combined survival rates when calculated as yield in grams/meter squared/day, than crayfish held in standing water tanks. Figure 11 displays, for Experiment II, the
Figure 10. Relative growth rates based on dry weights for subyearling crayfish in Experiment IV. Figures in parenthesis indicate mean percent dry weight.
Figure 11. The influence of velocity and photoperiod on subyearling crayfish yield in Experiment II.
Figure 12. The influence of velocity and photoperiod on subyearling crayfish yield in Experiment IV. Area below zero denotes negative yield.
Table 3. The effects of water velocity and photoperiod on the yield of subyearling crayfish in Experiments II and IV.

<table>
<thead>
<tr>
<th>Water velocity</th>
<th>Photoperiod</th>
<th>Experiment II (gm/m²/day)</th>
<th>Experiment IV (gm/m²/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Light-dark</td>
<td>0.147</td>
<td>-0.168</td>
</tr>
<tr>
<td>None</td>
<td>Dark</td>
<td>0.152</td>
<td>0.371</td>
</tr>
<tr>
<td>Slow</td>
<td>Light-dark</td>
<td>0.149</td>
<td>0.210</td>
</tr>
<tr>
<td>Slow</td>
<td>Dark</td>
<td>0.143</td>
<td>1.354</td>
</tr>
<tr>
<td>Fast</td>
<td>Light-dark</td>
<td>0.407</td>
<td>0.483</td>
</tr>
<tr>
<td>Fast</td>
<td>Dark</td>
<td>0.327</td>
<td>0.307</td>
</tr>
</tbody>
</table>
greatest yield for the fast-velocity tank. Figure 12 shows the highest yield for slow-velocity and fast-velocity tank subyearlings. A negative yield was calculated for the crayfish in the no-velocity tank, in Experiment IV. Photoperiod had no visible effect on the total yield for the crayfish.

**Temperature Profile**

The maximum-minimum daily temperature fluctuations for Experiments I, II and IV are shown on Figures 13, 14 and 15 respectively. The fluctuations in temperature for the first 24 days of Experiment I (Figure 13) are due to the water heater being accidentally connected into the day-night timer, turning the heater off at night and on during the day.

**Crayfish Lengths**

Length frequency for the crayfish used in Experiments II and IV are found in Figures 16 and 17. Crayfish from Experiment II had a mean of 5.24 mm with a range of from 3.5 to 6.8 mm. Crayfish from Experiment IV had a mean carapace length of 7.16 mm and a range of from 5.5 mm to 9.5 mm. Standard deviations were 0.671 and 0.771 for Experiments II and IV respectively.
Figure 13. Temperature profile for Experiment I depicting maximum and minimum daily temperatures.
Figure 14. Temperature profile for Experiment II depicting maximum and minimum daily temperatures.
Figure 15. Temperature profile for Experiment IV depicting maximum and minimum daily temperatures.
Figure 16. Length-frequency of subyearling crayfish used in Experiment II.

Figure 17. Length-frequency of subyearling crayfish used in Experiment IV.
DISCUSSION

Velocity Preference

Results of the first two experiments showed that water velocity had an effect on subyearling distribution throughout the artificial streams. In each tank with flowing water, there was a definite, visible migration to the slowest velocity available. In the slow-velocity tanks the subyearlings migrated away from 7 cm/sec and toward 4 cm/sec. In the fast-velocity tanks, the redistribution was away from 10 cm/sec and toward 7 cm/sec. Part of this preference, for the slowest velocity available in each tank, may be due to the fact that a small portion of food from each section was carried, with the current, to the end of each stream. A buildup of food in the last section may have attracted crayfish to feed there; however, there was always some food remaining throughout the stream at the next days feeding.

There appeared to be no effect on velocity preference from either photoperiod or stocking density. Orientation in the artificial streams was not influenced by photoperiod or density, but rather by water velocity.

The effect of depth on the distribution of subyearling crayfish, over the ranges used in these studies appeared to be unimportant. Velocity and not depth or photoperiod was the important parameter
in subyearling distribution. Based on these findings, shallow raceways with flowing water, appear to be suitable for culturing subyearling crayfish.

Survival Comparisons

Survival in the light-dark tanks was, in every case, greater than survival in the completely dark tanks. *Pacifastacus* spp. are nocturnal and thus tend to become lethargic and inactive during the day. This fact supports an idea presented by Roberts (1944). He reported that *Cambarus virilis*, also nocturnal, when exposed to illumination, released an eyestalk chemical that caused locomotor tendencies to become greatly reduced. Survival, therefore must be directly influenced by photoperiod. As day light exposure reduces the tendency to be active it reduces the one to one contact that leads to cannibalism. This would seem to explain the greater survival in the light-dark tanks.

The effect of water velocity on survival was less obvious. In general, survival was greater in flowing water than in static water. Both the density of stocking and the duration of the experiment had an effect on crayfish survival. Subyearlings, stocked at a rate of 30 per tank, ranged in survival from 60 to 90 percent over 17 days. This was twice the survival for subyearlings stocked at 120 per tank which ranged from 7-38 percent over 31 days. A comparison of
crayfish survival between the two experiments is difficult because:
(1) the first experiment lasted almost twice as long as the second experiment and (2) oxygen was added to the water in the no-velocity tank for the second experiment, and was neither added nor monitored in the first experiment. Despite the inconsistencies there is a major point that must be made concerning subyearling survival. The high mortality recorded for Experiment I, with the heavy stocking density of 120 per tank, appeared to eventually approach some optimal number of crayfish per unit area. This same number of surviving crayfish was approached by the subyearlings in Experiment II stocked at a much lighter density of 30 per tank. From Figure 5 it appears that this optimal stocking density lies between 50 and 100 subyearling crayfish per square meter.

Habitat Preference

Subyearling crayfish, in displaying a preference for leaf packs and 3-8 cm gravel substrate, confirmed two preconceived thoughts of mine on habitat selection. First, young crayfish require separate hiding places to escape contact. This was fulfilled in the hiding places provided by the 3-8 cm gravel. Second, the leaf pack was expected to act as both a food supply and as a secondary hiding place. The leaf packs were not only a direct food source but also a habitat for the tubificid worms which made up a major component of the
subyearling diet. Therefore, the substrate and food environments were met by the leaf packs and the gravel.

Virtually no animals were found in the sand or sub-3 cm gravel. Apparently the small gravel afforded fewer hiding places than the large gravel. Sand was used as a substrate, because it was thought that the ease of obtaining constant food and the ever present supply of sand to fill the statocyst organ would attract them. However, adequate hiding places were lacking. These habitat selections confirmed what was observed in the field in that very seldom were subyearling crayfish found in the upper stretches of the riffles where the gravel size is small or out on the sand away from cover. Only larger adults can safely venture out away from cover.

**Growth Comparisons**

The most striking differences in growth rates were in the no-velocity and slow-velocity tanks where the growth rate in the completely dark tanks were much greater than the growth rates in the light-dark tanks. These results were duplicated in both growth experiments for carapace length, wet weight and dry weight in the case of Experiment IV. Because crayfish are nocturnal feeders we would expect to find them feeding more at night than during the day. This could explain the higher growth rates in the dark tanks. A reversal of this pattern was found in fast-velocity tanks where growth in the
light-dark tanks exceeded growth in the totally dark tanks. These results were repeated in both experiments. Only changes in growth measured as carapace lengths in the first growth experiment showed less growth differences. An important point here is that the growth rates for all fast-velocity tanks were greater or nearly equal to all other growth rates. The fast-velocity created a condition which masked the effect of nocturnal feeding and allowed equal feeding to occur in either photoperiod.

There was a general trend of increasing relative growth rates as the velocity increased. However, the effects of photoperiod on subyearling growth were more pronounced than the effects of water velocity on growth. This was expected however, as the physiology and mechanics of feeding seemed to be more dependent on different light-dark conditions than on different velocity conditions.

The greatest growth rates for carapace length and dry weight were recorded in the light-dark and dark tanks with the fastest velocities. The greatest growth rate for wet weight was found in a dark, no-velocity tank. It is apparent from these results that fast velocities and total darkness are responsible for two of the three greatest growth rates; using wet weight, dry weight and carapace length as three ways of measuring growth.

A comparison of my growth data for subyearling crayfish provides a striking comparison with growth data reported by Coykendall
(1973). He reported a maximum of 0.598 percent increase in wet body weight per day for subyearling crayfish of the same species. My growth rates for wet body weight ranged from 2.083 to 4.793 percent increase per day. Coykendall also reported a maximum carapace length growth rate of 0.406 percent increase per day. Again my growth rates for subyearlings ranged from 0.735 to 1.843 percent increase in carapace length per day. I feel the difference in the two reported results lies in the experimental design. Coykendall (1973) held his crayfish in separate containers suspended in the recycled water and fed them to repletion. I feel, however, that his recycle system was not as conducive to growth. The much larger growth rates which I measured, I feel were due to the freer flowing stream-type environments.

**Yield**

The use of yield information with survival and growth data leads to constructive conclusions about subyearling performance. Yield was variable and may be due to a number of reasons: (1) in some instances the number of crayfish were so low that an addition or deletion of one extra crayfish would drastically change the yield for that particular tank or experiment; (2) total crayfish yield may not reflect the true capacity for that system to produce animal tissue, as dead and decaying crayfish and crayfish food cause an uneven and
unmeasurable oxygen depletion in the system and (3) there may have been some errors in either the weights of the animals or the measurement of stream area.

The yield of subyearling crayfish was quite conclusive, in that it showed a definite increase in yield for the flowing water tanks. These results, however, are inconsistent between experiments, as in Experiment II the greatest yield was found in the fast-velocity tanks and in Experiment IV the greatest yield was found in the slow-velocity tanks. The use of yield information is important as it combines the separate effects of survival and growth and thereby further emphasizes the effect of flowing water.

The yield results in Experiment IV were substantially higher than for Experiment II. Conditions for velocity and substrate were optimized for the last experiment and the yield, therefore, was expected to be higher.
CONCLUSIONS

It is very apparent that water velocity and photoperiod both had an effect on the survival and growth of subyearling crayfish. Experimental tanks with increasing water velocity consistently produced better growth rates, survival and yield for the subyearlings when compared to the no-velocity tanks. The optimum range appeared to be between 4 and 10 cm/sec. In the lower velocity ranges, photoperiod had a much greater effect on growth and survival than did water velocity as subyearling growth was greatly increased in the completely dark tanks while survival was greatly increased in the light-dark tanks. In the fastest velocity tanks this effect was reduced with there being little difference in growth rates and survival. I also found subyearling crayfish to prefer a habitat consisting of 3-8 cm gravel and leaf packs. When the optimal conditions of habitat, photoperiod and water velocity were combined I measured a yield of 1.354 grams per meters squared per day.

Based on the data presented I would encourage the use of the following basic designs in the culturing of subyearling crayfish: (1) a shallow, flowing water trough; (2) large gravel and leaf substrate; (3) reduced light; (4) the use of a living, non-putrifying secondary food source, such as tubificid worms and (5) stocking between 50 and 100 subyearlings per square meter.


