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THE EFFECT OF FOOD LeveL ano avrigioTiC

IN REARING LARVAE OF UCIOES CORDATUS LINNAEUS

(BRACHYURAN, GECARCINIDAE)

by

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July 1383,

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CENTER FOR ENERGY AND ENVIRONMENT RESEARCH

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July 1983,

Research was conducted at the Center for Energy and Environment Research under the auspices of Oak Ridge Associated Universities Summer Student Participation Program

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ABSTRACT

Larvae of Ucides cordatus Linnaeus, the mangrove crab, were reared

In 9 flow-through culturing apparatus under two different feeding

levels and the condition of antibiotic or non-ant idiot

survival

under atl conditions was low, yielding statistically insignificant

results, but the trends showed better larval survival under antibiotic conditions, Little survival difference was noted between different feeding levels. Suggestions on culturing brachyuran larvae in the future are presented as part of this report.

A short exploratory bicassay wis also conducted. Usi

19 the Flow

through system, 2 mixture of brachyuran zeae were exposed to three concentrations of primary-treated sewage for 24 hours, and a 24 he LC5O

was éetermined through analyses of the data.

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' Hater quality data for 24 hour range find

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Pace

Cut-away view of one aquarium used in the culty

oF uel WENO eee ener nes 6

Set-up for one effluent concentration in the explora-

tory bioassay with brachyuren soeae, ss se Poon

Mortality of Ucides cordatus larvae with tine, at feed!

level 600 (600 Artenfa salina fed to each group of

twenty animals OF FWFD ee ee ee ee TS:

Nortality of Ucides cordatus larvae with tine, at feeding

eve! 1000 (1000 Artemia salina Fed to cach group of

twenty animals on FD eet ee ee +216

Semi logarithmic plot of brachyuran bioassay results

showing the 24 hr C50 value?. 9

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INTRODUCTION

The culéui

19 Of erab larvae in the laboratory has been the source

Of a great deal of research. Brachyurans, including the land cr

Kelp crabs and shore crabs have received the greatest amount of attention, possibly due to their economic value. ?Hany species of brachyuren

larvae have been subjected to salinity/tenperature combinations

(Cost low and Bookhout, 1968; Young and Hazlett, 1978), different feeding regimens (Anger, Klaus, Dawirs, Anger and Costiow, 1981), and rearing

in @ voriety of environmental systens. Sone expe

iments are designed

to examine the physiology of the larvae (Levine and Sulkin, 1979), or

describe the different stages in larval development (Costlow, et. al

1968), or examine larval behavior (Sulkin, Van Heukelem, Kelly, and Van Heukelem, 1980) or investigate the possible advantages of @ new + 1960; Buchanan, Myers, and Caldwell, culturing system (Costlow, et. al 1975).

This research investigated possible effects of antibiotic and feeding level on the survival of larvae of the mangrove crab, Ucid cordatus ised in the laboratory. The larvae were reared in a specially designed culturing set up from first zoea to negalopa, at which point the experiment was terminated. Larvae were held under two

different feeding levels and the condition of antibiot

treatment or

non-antibiotic treatment. The same culturing system was later used for a 24 hr range Finding bioassay using a mixture of species of crab larvae to assess toxicity levels in Puerto Rican primarily treated sewage. Data From the bioassay are presented to establish critical

Himits for future bioassays with crab larvae.

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LITERATURE REVIEW

General culture technique of brachyuran decapods dictates the use Of Artemia salina nauplii as the sole source of food (Roberts, 1975).

Brine shrinp are easily hatched in a pe:

1d of 48-72 hours, and are

smal! enough in the nauplius stage to be handled by the mouthparts of

the crab zoeae, Experinentat

mn with the xanthid crab has shown the

?optimum feeding level of Artemia to be a food density of five nauplii

Per ml of seawater (cited in levine, et. al., 1973). Feeding reginen

Js variable from report to report, ranging from three days weekly

(Buchanan,

als, 1975) to daily feeding (Costiow, et. al., 1968;

+ 1979).

Levine, et. at.

Srachyuran larvae have been raised in a variety of rearing systems,

both static and free-flowing, most systems surveyed being static.

Young, et. al. aL, (1981) separated zoeae into.

(1978) and Anger, et,

individual compartments of large trays, which were kept in environmental chambers under constant tenperature and a fixed light schedule, Costlow,

et. al. (1968) and Buchanan, et

(1975) grouped larvae 10-20 per

container: Costiow used an Ederbach variable speed shaker to neintain

Taevae, while Buchanan utilized a reverse-flow Flowing water system.

al

Finally, Levine (1979) ana sutkin, + (1980) grouped up

to 200 larvae in single container and maintained them under constant

temperature and light conditions

Antibiotics have been widely used in culturing marine invertebrates.

Im the Iterature surveyed, limited information was found hich compared

the effect of antibiotic against 2 non-antibiotic control. As efter

in Sulkin, et

(1980), egg end larval survival are improved,

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no effect on larval behavior, through the use of ontibioties (unpub=

Hished data), suikin's set-up used antibiotic continuously in the

system, a5 contrasted with antibiotic axenization, 0 process whereby

e905 and larvae are exposed to missive doses of ani

s for brief

Periods of tine, The advantage of axenization is that long-term effects of antibiottes, such as larval growth inhibition, are no longer eritical factors (O'Agostino, 1975)

4m addition to reporting the findings of the culturing experiment , thls paper also presents the results of a short exploratory bioassay conducted with @ mixture of crab zoeae of several species, Bioassays are effective means to determine the toxicity of industrial wastes to % particular organism, The purpose of this 24 hr bioassay was to determing 8 concentration of sewage effluent which is lethal to 50% Of the test organisms (a 24 hr LC5O). Range finding, or exploratory bioassays function to determine concentrations of effluent which will de critical in future bioassays of longer endurance. One concentration in a range Find shoutd kill all of the test organisms (a maximum dilution to be used), and one concentration should kil! no organisms (@ minimal

dilution to be used)

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MATERIALS AND HETHODS

Larvae culturing

A single ovigerous female mangrove crab was collected from the

wangrove-tined channel of Joyuda Laycon, a tropical lagoon on the west

coast of Puerto Rico. The spe:

n was taken to the laboratory, whe!

Its abdomen was removed, intact with pleopods ond eggs. Pleopods were

removed with their clusters of eggs intact and immersed in two successive

water baths (250 ml) for one minute in each bath. Wash water (sali

ity

35 p.p.t., and temperature 2741 °C) had been passed through a 6lemieron Filter. Gentle swirting of the eags, by grasping the plecpod stem of each cluster, allowed debris to be washed free. Ninety percent of the 995 were then transferred with forceps to an antibiotic bath (5.5 mp Nitrofurazone, 2.3 mg Furazolidone, and .18 mg Hethylene blue in one

liter of water), and 10% were transferred to one

fer of non-ant.

jotie

water, Ieersion time was 30 minutes, The antibiotic axenization method was based on Peltier (1978), which suggested 3-5 mg of nitrofurazone/ 1 for 30-60 minutes immersion. The antibiotic mixture used throughout the experinent was 2 commercial brand marketed as Furan-2, According te manufacturer specifications, each Furan-2 capsule contains 60 mg nitrofurazone, 25 mg furazolidone, and 2 mg nethylene blue, The capsutes 150 contain about 600 mg of a filler, so to partition a given mass of nitrofurazone, @ simple proportionality using the mass of the entire

capsule contents was used.

Following washing, the egos were placed in the culturing system.

?The system consisted of two identical set-ups, each of the fol lowing

design. Four, 502 ml plastic, circular containers were housed in a

---Page Break---

Fivergalton insulated aquarium. Each container had approximately 35%

wall area and $5% Tid area removed and covered with 202-mi

ron mesh,

Which was secured with silicone rubber, This allowed enough water to

Elrculate through each container so that it would turn over in volume

Jn less than an hour with 250 ml/minute flow into the aquartum,

Containers floated, so they were anchored to the hottom with plastic

bars. Water Flow to the system was maintained by @ single jet to the

bottom of the aquarium (Figure 1). The system was located in an out=

door sheltered area.

Ten percent of the antibiotic eggs were transferred to containers

1-4 and placed in aquarium "A", These four containers were treated with antibiotic through the remainder of the experiment, and they were ateays maintained in the same aquarium. Non-antibiotie eggs went to containers 5-B in aquarium "6", and the surplus antibiotic eggs went to @ five-gatlon Flowing water batch culture.

Twenty-four hours later, containers were examined, and al Uunhatehed eggs and tarvae were renoved to leave only 20 larvae per Container. Since there was such limited survival in the non-antibiotic containers, ai! larvae for these containers were drawn from the bateh culture. Thus all larvae in the culturing experiment hatched From antibjotically axenized eggs. Ove to lack of Artemia, first feeding Of the zocae and Formal initiation of the experiment were delayed to 4B hours after the initial introduction of the eags to water. At 4B hours, tine zero for the experiment, larvae were counted in each container. If @ container held less than 20 zoeae, it was re-stocked

from the batch culture, Since eggs were continually hatching in the

---Page Break---

fed in the culturing of Ucides

?ontainers have been rensvede?

---Page Break---

batch culture up to this tine, beginning larval age could range from

@ few hours 6 almost WB hours.

Larvae were fed three times weekly (MMF), and on these same days,

they were transferred to clean containers and antibiotically axenized,

If From the appropriate group. Containers were renoved from the aquarium

and carefully rinsed down before and after opening. Larvae were

individually transferred, via a mouth pipette, to» clean container,

and during this process, larval live and dead counts were made with

missing lorvae counted as dead. Clean containers sat in two enameled

trays, each holding two liters of water, one with antibiotic and the other without. The latter served as a control for this part of the

?experiment. Due to the mesh walls of the containers, water from the

trays circulated into them, filling them to approximately 80 ml
Inmersion was for 40 minutes ond was done outside to keep the bath temperature close to that of the culture temperature. The dirty

containers from which the larvae were removed were washed in an Alconox

solution, followed by @ thorough tap, distilled and sea water rinse.
Antibiotic levels used ranged From 4,08-5.51 mg nitrofurazone/!, averaging 4.65 mo/I. Correspondingly, average furazolidone and

methylene blue concentrations were 1.94 mg/I and .156 mo/l, respect!

rely.

Following inners:

+ the containers were floated In the aquarium
Until fed, then capped and secured to the aquarium bottom. Feeding was at two levels: one group of zoeae received 1000 Artenia/container/ feeding session, while the other group received 600 Artemia/containter/ feeding session. Each Feeding group included two antibiotic and two on-antibiotie containers, thus providing two repetitions for each

experimental condition.

---Page Break---
The Art mia were cultured in ono-gallon nasen jars in roume temperature ater (27 °C; salinity 35 p.p.t.) under gentte ae-ation,
On 8 feeding day, 2 small Flucrescent lanp was placed near the 48-96 hr culture, and within 15 minutes, lerge numbers of nauplii and prezoeae were collected via pipette. Two, one-al subsamples were taken from the Jar of collected brine shrinp and counted. Using the shrinp/mt

estimates from the subsamling, apprcpriate volumes from the jar of Artemia were determined for feeding.

Since the set-up was outside, it was subject to temperature Auctuations induced by the anbient temperature, Thermograns made With a Ryan herrograph on two separate occasions (Four days each)

showed a terperature of 27.842.8 °C on the First occasion and 29.54.9 °C

fon the second, There was a definite thermal cycle present in a 24 hr

Perio. Since the Flow into the aquariums was unregulated, it also

varied greatly. Aquarium "A" Flow was 229461 mI/min., and aquartum Flow was 248494 mi/min. Salinity of the water, which was unfiltered, ranged from 35-35.5 p.p.t.

Data collected on the larvae were for mortal

ry and appearance of

megatopa, the first post-zoeal stage. When 9 megalopa appeared, it was

Femoved from the experiment and counted as a larva that survived.

A-24 hr range Finding bioassay was conducted in the flow-through system, Zoese were field collected in a half meter plankton net tow (202 micron mesh) from Joyuda Lagoon and held in the lab for 24 hours without addition of food. ater quatity in the lagoon at the tine of

collection was 28.2 °C, 33 p.p.t., pH=B.2, and 0.0,

<4 p.pam. Ouring

---Page Break---

the 24 hr holding period, a Flow of bioassay dilution water (approxi mately 150 ei/min) was maintained to the holding bucket, The quality Of this water appears in Table 1 under "Control", The collected zoeae

were a mixture of species, al! of the superfamily Brachyrhynca,

The bicassay set-up consisted of four pairs of Five-gallon aquariums, each pair receiving one of four concentrations of toxicant, The toxicant was primary? treated sewage outfall from Arecibo, Puerto Rica, Cach squarium held one flow-through contsiner of the sane design as mentioned in the previous section, This set-up was in accordance with APHA, AVNA, and WPC (1576) which indicates that true replicates must be mode in which there is no water connection between the replicate containers.

?The concentrations of effluent used were 0 (control), 2.5, 5, and 10% by volume. Dibutions were made by adjusting the effluent Flow rates with

peristaltic pumps which drew the toxicant from $0 9

ton barrels. The

toxicant was mixed

Fh dilution woter, Flow rate also regulated with
Peristaltic pumps, outside of the aquariums in Y-connectors and a length of tubing (Figure 2). Mater quality was monitored twice during

the study (Table 1).

Zoeae were dravm from the holding bucket with a mouth pipette and transferred to containers. Thirty-five zoeae of approximately the same size were placed in each container and fed a mixture of Artemia nauplit (200) and eggs (1400) before being introduced to the bioassay. Twenty-four hours later, containers were renoved, rinsed free of effluent to stop the bioassay, and poured Into counting chambers. Dead and Vive counts were made under low nacni fication, with missing larvae

counted as dead. Dota were plotted on semilogarithmic paper for the

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TABLE 1. Mater qual ity data for 24 hour rang

Ddrachyuran zeae.

Puerto Rice,

Finding bioassay on assorted

Toxicant was prinary-treated seuaye From Arecibo,

Trea:rent Dissolved

repe'Telon oxygen Salinity Tg

aunbar 3 pest Cine

contro} 1 55 2.43 ero

Contra 385 2128 ere

3 360 27:43 ero

?3 33 273) ere

50 : 350 28:13 here

33 2 x0 28:28 tere

2 ws 212 tere

ve 2 35.0 7838 Here

const 1 36.0 28.622 hours,

Eenerot 2 36.9 28:35 bu hours

3 i 30°0 27:67 4 hours

?0 2 i 30 27°30 bh hours

50 i : 35.0 28:10 24 hours

30 2 : 3 28.18 2 hours

zs : ? 5.0 2812 Bh hours

25 2 33 B10 35.0 28.12 2 fours

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purpose of graphical interpolation. This plotting method is used to estimate the 24 hr LCS0 by plotting concentration of effluent (& by volune) on a logarithmic scale against percent mortality on an arithmetic seale. & straight line is drawn between every two consecutive Points, and the LC50 concentration is estimated from the Tine connect ing the points which bracket the 50% mortality level on the arithmetic scale.

The LC50 sewage concentration is simply read from the tog seate (APHA, etal, 1976)

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ResuLTS

Larvae culturing

The culturing experinent endured for 9 period of 24 days ofter

whieh a11 larvae had either died or reached megatopa (Table 2, Figures

Band 4). Data for repetitions of a given condition were summed (thus

49 zoeae at tine vero for each condition) and analyzed using 2X2 6 test

for de.

tion From random expectation (Sokal and Rohlf, 1969). The

results were shown to be statistically insignificant at the 95% confidence

level. Since this is the case, results will be presented as trends,

The onty graup of zoeae which produced megalopa were the anti

bioticatly treated larva

4m adéicon, at day 13 only two non=

ancisfotle animals were alive compared to 13 living antibiotic eninals

This Mlustrates the trend that, except for day 9, there were always

more antibiotie than non-antibiotic animals alive. On day 9, there were equal number of animals alive From both conditions. A total of five egalopa were produced from the antibiotic animals, making final

mortality $48, conpared to 100% mortality for non-antibiotic animals,

Feeding trends are not so obvious. The antibiotic animals receiving 1000 shrimp per meal produced three megalopa, and the group receiving 5600 shrimp per meal produced two. The megalose appeared first in the 1000 group, but no analysis can be applied here due to the variability Of terval starting age and infrequent observat ions,

Bioassay

The results for the range Finding bioassay appear in Table 3. The

Fesvlts for each -epetition were sunned (thus 70 aninals per experimental

condition) to arrive at the percent mortality figure. The control shows

B

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TABLE 2.

anvae

FeD 609

s

Mortality of cides cordatus with tim. Mortality is shown for each

repetition and ?uimed to calculate . mortality (out ef UD organieas)

The tine in days appears In Fractions because it represents fio to

the closest hour when dead aninals were counted

The antibior ie

animals were alnays treated ané fed defore the nonvantibiotic. animals

?Asterisks

Were (thus the diserenancies of tims between arsups)

Feorosent megelopas

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ort 0 '12.5172.5 145 63.3 | 3s |e7.5 Ls0_| 52.5

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FE0 600

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FED 1000

Artemia

__NoN-ANTIB1OTIC LARVAE

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28 ~ -

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es

% Antibiotic 2080

+ Won-antibiotie zoee

- is

8 18 12 M4

TIME (LAYS)

Figure 3. Wortality of Ucides cordatus larvae with tine, at feeding tevel 600

?act fed (o each group of twenty animals on MMF)

Fach curve represents the sum of dead animals trom both replteaies,

Asterisks represent megaleoa at the indicated day.

15

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Figure 4

1

Joe

to.

no

4

4 Antibiotic 208s

+ Nonvantibiotie 200

bee dt

4 6 8 If 12 14 16 18 28 22

COAYS >

Mortality of Ucides cordatus larvae with tine, at Feeding level 1000

(1000 Artemia saTina Fea to cach group of tuenty aninals on H¥F)

Each curve represents the sum of dead aninals frow both replicates.

Asterisks represent megalopa at the indicated Guy,

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TALE 3. Mortality of brachyuran zoeae involved in the 24 hour range Finging

bioassay. Percent mortality represents the tota! miler of dead ovae.

From each concentration (nurber dead out of initial 70 tervec), Toxteont

Used was primary-treated sewage from Arecibo, Puerto Rico.

Concentration of Effluent (% by volume)

ote 3 7

Rept Hepes tap 1 Re

ra |

Casual ° ° ° 5 ° w |»

sunor | I

Bers ° 5 i ?

f /

fontury | hit wa on

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1008 survival, ond each successive effluent concent

shows

decreasing survival, with mortality at 10% effluent approaching 1003.

Data were analyzed using graphical interpolation (Figure 5), and the

24 hr (C50 value obtained was 6.723 effluent.

---Page Break---

Figure 5.

ein

Concentration of Sewage (% by volune)

Semilogarithmic plot of brachyuran bioassey results showing

the 24 hr LC50 value

---Page Break---

Discussion

Larvae culturing

Even though the results of this culturing experinent are statis
tically insignificant using a G test, the trends observed are worthy
Of note, The fact that only antibiotic zoeae reached megalopa ond
always had greater munber of animals alive at 2 given tine suggests
Positive results. Furthermore, the hatching of non-antibiotic eggs
was visibly poorer than antibiotic hatching. 1 would definitely

reconmend the use of antibiotic in cultu

19 crab larvae. Further
research should investigate the effects of various antibiotics and
their optimum concentrations to further improve survival of larvae.

Through Hiterature review, several distinct flaws of this research
became apparent. Feeding problens were the major Flax, Followed by
factors beyond experimental control inherent in the laboratory water
system, and possible handling technique problems,

Feeding problems were two-fol

Primarily, an initial starvation

Period could have accounted for @ large percentage of the mortality.

In experiments with several species of brachyurans, initial starvation

Periods of 24-48 hours had profound effects upon larval survival, even

If z0eae were re-fed adequately following starvation (Anger, ef

1981). Starvation during the first 48 hours increased mortality before

and during molting from zoea 1 to z0ea 2, and also increased mortality

of first stage 208:

after the first re-feeding. It also significantly

lengthened the first zoeal stage and the length of development to

egalopa by slmost 50%. My experinent started 48 hours following egg

immersion, so zoeae could have been starving for any period of time up

---Page Break---

to 48 hours prior to their First feeding. Clearly, initial starvation of larvae can have a very detrimental effect on the outcone of the experinent.

The second feeding problem was insufficient food density. Food levels were based sonexhat on Buchanan, et.

al. (1975), but later

Niteracure review indicates that these levels were probably too low. As cited In Levine, gt. al. (1973), the optimun density of nauplii for the xanthid crab was five nauplii/al, and these were daily feedings.

The 1000 level feeding brought food density to two nauplti/ml, and the 600 level feeding was close to one nauplius/ml, and feeding was only three tines weekly, Underfeeding probably explains for the disappearance Of Sone animals through cannibal ism (witnessed on occasion) and perhaps also significantly contributes to the high mortality through starvation.

The second major source of problens cane from the

ladequacies

Of the laboratory water system. On one occasion, water flow had been

accidently shut-off and the water had completely drained From aquarium

+ leaving very little water for the zorae. Secondly, the water is
not sterilized, so it may be rich in organics. The storage tank in
which systen water Is held may accumulate debris and organic matter,
and coupled with the worm temperature of the water, this could provide
42 favorable environment for bacterial growth. Since there were no
Westerilizers or water filters smaller than 202 microns, bacteria
were possibly @ factor in the high larval mortality. When dead zoea
were examined, they were loaded with ciliate bacteria grazers such as
Uronena spp. In the literature reviewed, most set-ups relied on

thoroughly filtered sterilized water for their systens.

a

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The third source of problens may heve been in handling technique. torvae were transferred individually in 9 Pasteur pipette from container to container. The problem with this technique may be abuse to the z0ea through over handling. The zoea's defense to disturbance is rolling Into a ball, but its dorsal spine is stil! exposed. Fracturing the spine coulé make the larva very susceptible te infection. The rim of the pipette's bore may have damaged sone larvae in this manner,

From this research, the most inportant information gained was Probably, ?how to do it better next tine."! For future culturing attempts With crab larvae, | can make the fo} loving suggest ions:

1. Use antibiotic axenization for eggs and larvae, perhaps on @

sched,

Of four times weekly. Three tines weekly still allowed a good number of bacteria to thrive as evidenced by the abundence of bacteria grazers on the dead organtsas

2. Feed larvae the suggested density of five nauplit/ml/day.

This shouldensure limited starvation and cannibalism, Feed Artemia in the maupiius (48-72 hours) stage. They are nutritionally more valuable then prezoeae and are easily handled by the crab zocae.

3). Monitor hatching closely, keeping the nunber of eggs/container less than 300. When hatching is first observed, introduce food to the containers to avoid the lethal effects of early starvation.

4. Westeritize and filter system water so that the bacterial problen can be minimized,

5+ Conduct pre-experinents to determine recoverability of zoeae

With different handling techniques (pipetting, pouring)

---Page Break---

sssay,

The results of the range finding are useful in evaluating bioassay ethods for brachyuran larvae, and determining their sensitivity to

Puerto Rican sewage toxicants. They indicate that the maximal bioassay

?seuage concentration used in future brachyuran bioassays with Arecibo

stuage effluents probably need not exceed 10% by volune. In addition

te Information concerning sewage concentrations, the handling technique

of pouring the flow-through container contents into a counting chanber

was proven to be very effec

fe in recovering the organisms from the

containers. This is important infornation because hand) ing

assay

organisms vie a mouth pipette is potentially toxic to the experimenters,

Recoversbility was 100% in al! contro! containers indicating that holding

methods don't thenselves contribute to larval mortality,

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