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Title: THE EFFECTS OF TRITIATED SEAWATER ON DEVELOPMENT OF THE GOOSE BARNACLE POLLICIPES POLYMERUS

Abstract approved: Redacted for privacy
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The purpose of this study was to determine the effects of tritiated seawater (HTO) on selected developmental stages of the goose barnacle, Pollicipes polymerus. Barnacle embryos were cultured in Millipore cytology monitors with HTO concentrations of 0, 10^{-5}, 10^{-3}, 10^{-1}, and 10^{1} μCi/ml. After 22 days, hatching was complete and the larvae and unhatched eggs were fixed in formalin. Criteria used in evaluating the effects of HTO on development were morphological abnormalities, a "molting index" of stage I nauplii larvae and the mortality of eggs. Effects were seen at the lowest concentration when evaluated in terms of abnormalities and the molting index. Mortality, or viability of eggs, showed no clear dose-effect relationship.

The Effects of Tritiated Seawater on Development of the Goose Barnacle *Pollicipes polymerus*

by

David Thomas Abbott

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I. INTRODUCTION

Radioecology of Tritium

Tritium, an isotope of hydrogen whose nucleus contains one proton and two neutrons, is an abundant, widely distributed radionuclide. It is produced naturally by spallation reactions involving cosmic ray interactions with ^{14}N, ^{16}O, and ^{2}H in the upper atmosphere. Tritium is also directly accreted from the sun (Evans, 1966). Virtually all the tritium so produced reacts with oxygen to form tritiated water, variously symbolized as THO, HTO, or ^{3}HOH. Although estimates of the world inventory of natural tritium vary from nine to 275 MCi, a recent value of 28 MCi seems reasonably accurate (UNSCEAR, 1972).

Large amounts of tritium have also been produced artificially in association with human activities. Although nuclear fission bombs produce some tritium, the largest contribution has been made by the atmospheric testing of thermonuclear fusion (hydrogen) bombs when tritium is produced as a result of neutron capture involving ^{6}Li and ^{2}H (Peterson et al., 1969). Most of this tritium is "burned" in the ensuing fusion reactions, but large quantities escape into the atmosphere. At least 1700 MCi have been released to the environment

as a result of weapons testing. Since this source has been greatly reduced, interest is now centered on production by fission reactors.

There are two major modes of production in nuclear reactors. Tritium is produced as a result of fission reactions with one fission in every 10^4 reactions producing a triton. Since this occurs in the cladded fuel rods, most is not released until the rods are reprocessed (Logsdon and Hickey, 1971). The other mechanism in reactors involves neutron activation of substances in the reactor, mostly ^{10}B, ^{6}Li, and ^{2}H in the cooling water. This tritium, in the form of HTO, is released from power reactors to the environment. It has been estimated that by 1995 the nuclear power industry's contribution will exceed that which remains from weapons testing (Peterson et al., 1969).

The coastal and nearshore areas of our country are becoming attractive as locations for building power reactors because of the huge volume of water available as a coolant and diluent. Because of atmospheric input (primarily rain and snow) rivers have elevated levels of HTO. In addition to direct input from the atmosphere, the mixed layer of the ocean receives HTO from rivers. Additional elevation of HTO levels often occurs when nearshore waters mix poorly with offshore waters (RIME, 1971). Potential sitings of coastal or nearshore nuclear power plants, which would result in a further increase in HTO levels, have caused some concern about

effects on the littoral zone environment.

Biological Effects of Tritium

A good benchmark for discussing the tritium levels used in studying biological effects is the maximum permissible concentration of ^{3}H in water (MPC_w), 3×10^{-3} μCi/ml. Since the primary concern of man is to determine possible effects on himself, mammals have been widely used in determining radiation effects from HTO. The lowest concentration at which negative biological effects were found involved female mice maintained on drinking water containing 1.3×10^{-1} μCi/ml of HTO that gave birth to female offspring which had a significantly reduced number of primary oocytes (Dobson and Cooper, 1974).

Studies on fish have usually been short in duration and consequently the results have failed to demonstrate significant effects at low HTO levels. However, Strand and Fujihara (1973) showed that the immune capacity in rainbow trout raised in 1×10^{-1} μCi/ml HTO was reduced.

Generally, the only studies to show low level effects were those that involved a truly chronic exposure where the organism had a chance to equilibrate with the environment. More often than not, equilibration did not occur (Uchiyama, 1973; Ichikawa and Suyama, 1974). Not only does chronic long term exposure permit the

organism's body water to equilibrate with the environment, but tritium will also be incorporated in organic molecules such as DNA (Smith and Taylor, 1969; Ueno, 1974). The long term genetic consequences of such tritium incorporation are unknown.

Research Animal

The animal selected for this experiment was the goose barnacle, Pollicipes polymerus, which occurs worldwide in rocky, exposed, intertidal areas (Cornwall, 1970). It is an ecological dominant in the Mytilus-Pollicipes-Pisaster (mussel-barnacle-starfish) community (Ricketts, Calvin, and Hedgpeth, 1973). The clustered adults play an important role as predators, scavengers, and omnivorous general feeders (Howard and Scott, 1959). The hermaphroditic adults (Figure 1) are believed to begin breeding when the water temperature reaches 12.3° C, and their breeding season lasts from May to December in California (Hilgard, 1960). The eggs are fertilized internally and then extruded into a pair of sacs, the ovigerous lamellae, located on both sides of the mantle cavity. There are approximately 50,000 to 60,000 eggs per brood. A mature adult goose barnacle probably raises four to six broods a year, and about 50 to 60% of the mature adults can be found to contain ovigerous lamellae during the peak of the breeding season. Embryos contained in the ovigerous lamellae of an individual

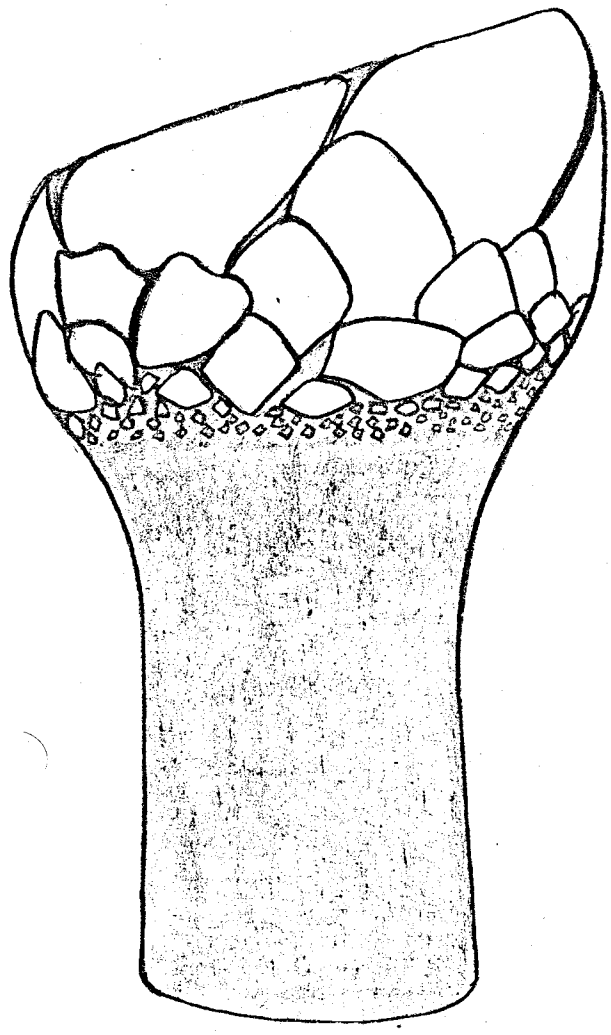

Figure 1. A solitary adult goose barnacle.

start development synchronously (Barnes and Barnes, 1959a), but the adults in a cluster are not synchronized breeders. The protostomous, yolky zygote undergoes unequal holoblastic, spiral, determinate cleavage (Anderson, 1973; Barnes, 1974). The development rate of the zygote and embryo is temperature dependent, and hatching and release of nauplii larvae normally occurs about one month after extrusion (Barnes and Barnes, 1959b). Six naupliar larval stages are followed by a cypris larva which about one month after hatching is chemically attracted to existing adults and settles nearby. After settling, the cypris larva metamorphoses into an adult. Methods for culturing the larvae, which are probably an important food source in the littoral zone, have been previously developed (Barnes and Barnes, 1959b).

Purpose of Study

Marine invertebrates have been largely ignored in HTO studies. An attempt was made to determine the effects of HTO on developing Pacific oysters (Crassostrea gigas) but technical difficulties in culturing precluded any meaningful results (Uchiyama, 1973). No attempt has been made to determine the effects of HTO on any other marine invertebrate. In view of this lack of knowledge and the aforementioned increasing world inventory of tritium, it seemed desirable to look at a marine invertebrate of either economic or ecological

importance. Since the "dirtiest" power reactor in the country, the San Onofre-1 plant, is located on the Pacific Coast (NUREG, 1975), I decided to study an intertidal animal whose range covers most of the West Coast of North America. The primary objective was to study the effects of HTO on the larval or early developmental stages of such an animal, since these are usually more radiosensitive than more mature stages. A relatively long period of development was also desired in order to assure equilibration and incorporation of HTO into the animal. The overall purpose of the study was to rear a marine invertebrate in various concentrations of HTO and determine the effects relative to mortality, developmental abnormalities, molting success, and chromosomal aberrations.

II. MATERIALS AND METHODS

Collection of Eggs

Eggs contained in the ovigerous lamellae of ripe P. polymerus were collected at Yaquina Head, Oregon. An abundant barnacle population exists at this location which was readily accessible at low tides. Additional populations may be found at Seal Rocks, Yachats, and other exposed rocky locations, provided there is not too much sand present. To obtain eggs, barnacles were opened in situ by severing the adductor muscles with a small pair of scissors and looking inside the mantle cavity. Ovigerous lamellae were removed with plastic forceps and placed in 25 ml glass vials which were filled with synthetic seawater containing antibiotics. The vials were then placed in a styrofoam container filled with cold (ambient) natural seawater and transported to Corvallis. Collection was carried out under stipulations of a Fish Commission of Oregon scientific collecting permit.

Preparation of Seawater

The seawater was prepared by mixing Instant Ocean synthetic sea salts with tap water. The resultant hydrometrically determined salinity was 34 ppt (Zerbe and Taylor, 1953). Penicillin-G and streptomycin sulfate (Sigma Chemical Company of St. Louis) were

added to a concentration of 100 ppm each. The fungicide mycostatin, trade name Nystatin (Sigma Chemical Company), was prepared as a stock solution with distilled water and added to the cultures daily to an estimated concentration of 20 ppm. Twenty-five mCi of tritiated water, obtained from New England Nuclear, was mixed in serial dilutions with the prepared seawater. HTO concentrations of 0, 10^{-5}, 10^{-3}, 10^{-1} and 10^{1} μCi/ml were used in this study. The solutions were stored in capped flasks and kept in a water bath.

Culturing Apparatus

The major components of the culturing apparatus were: a double water bath; a cooling unit; culture chambers; an aeration system; and an evacuation hood. Figure 2 shows the basic components. The inner water bath (d) was a small, two gallon glass aquarium which was supported in the outer water bath (b), a 15 gallon aquarium, by rubber coated test tube racks (c). This double water bath was used to minimize decontamination problems in the event of leakage from the culture chambers (a). The HTO and control solutions were stored in Erlenmeyer flasks (e). A Hush III pump (f) connected to surgical tubing provided the air supply. A Westinghouse model WNC10KG1 cooling unit (not shown) coupled with a submersible pump (g) maintained a temperature of 13±1° C throughout the experiment.

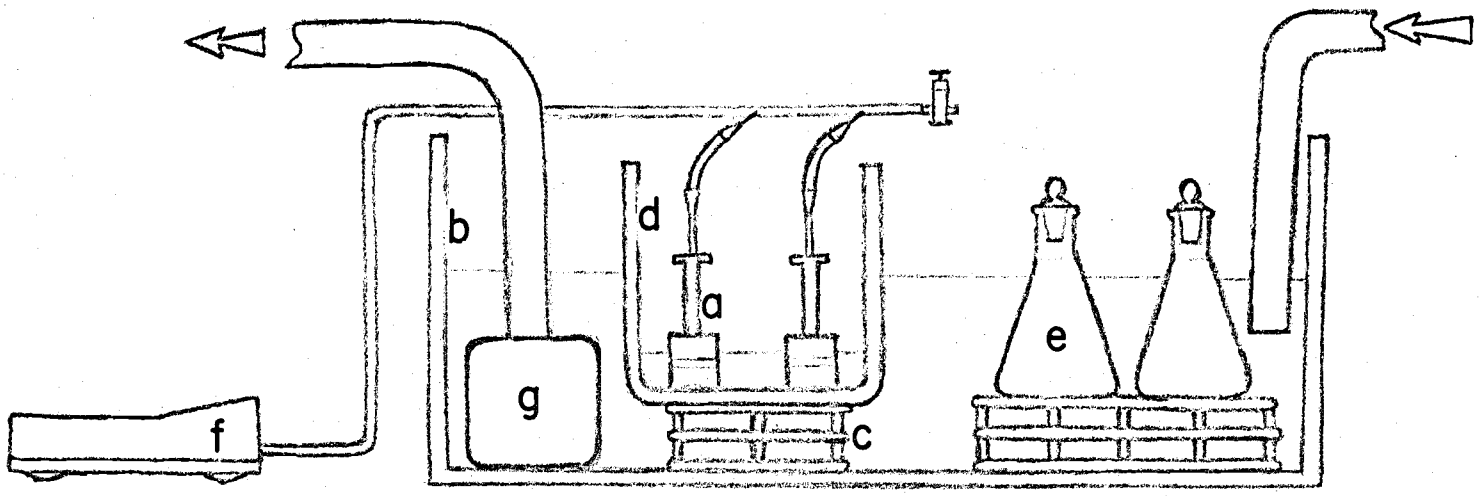

Figure 2. The apparatus used in the experiment. Consult text for explanation.

The egg masses were sliced into wedges of approximately equal size and placed in the culture chambers. Water, with drugs, was changed daily and, although the chambers had a volume of 10 ml, only five ml of solution were used to prevent the water from bubbling out. The culture chambers were placed in the inner water bath for the 22 day duration of the experiment.

The culture chambers (Figure 3, a) were clinical cytology monitors, Millipore MSMP 037 HO. These chambers have plugs (f) which can be removed for changing water and inserting a bored out one ml disposable syringe barrel (b) which is part of the aeration system designed to prevent escape of HTO. To move air from the surgical tubing to the culture chamber, a removable device (c, d, and e) was constructed. A disposable hypodermic needle (d) was glued onto a capillary tube (c), a short piece of rubber tubing (e) was glued to this and another needle (d) was attached to the free end of the tubing. This last needle could be plugged into the air supply (the surgical tubing) or disconnected. The capillary tube (c) could then be inserted down the syringe barrel (b) into the culture chamber (a) to provide gentle aeration. Except during the daily water changes, aeration was continuous in the chambers.

The culture chambers were examined under the stereo dissecting microscope daily to determine if hatching had occurred. The entire apparatus was placed in an evacuation hood (not shown) to vent off

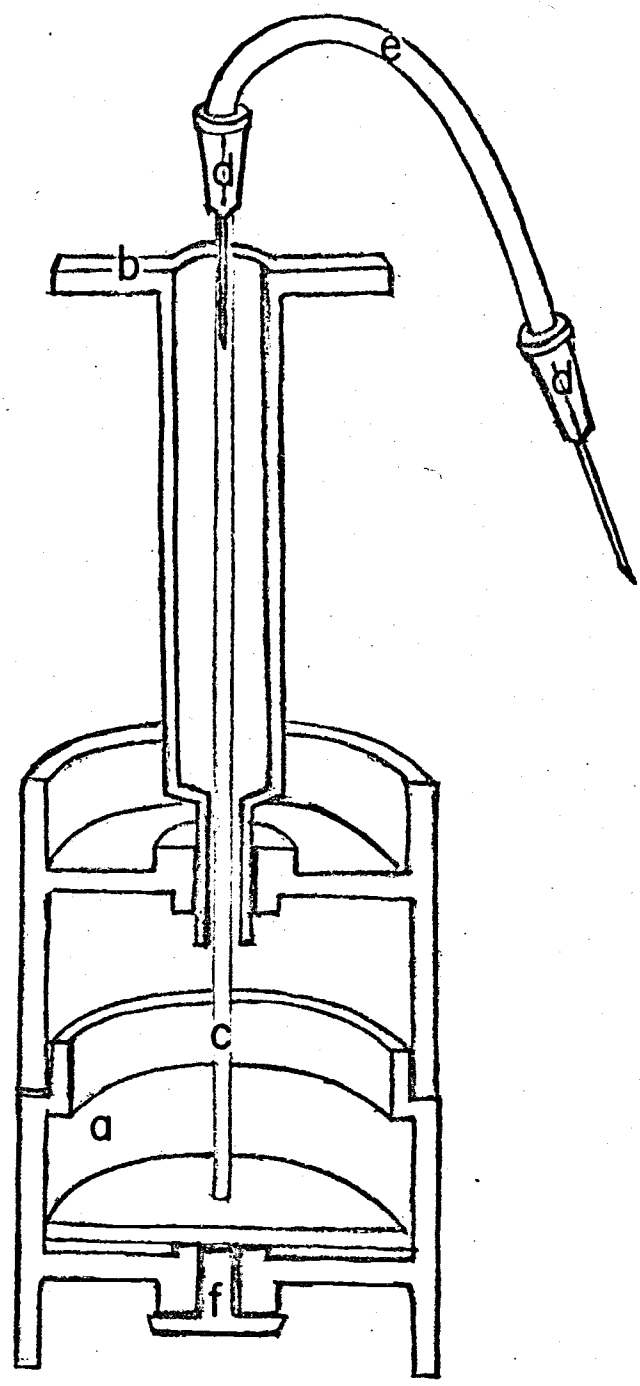

Figure 3. A semi-diagrammatic cross section of a culture chamber with aeration tube inserted. Consult text for explanation.

escaping tritiated water vapor. Water changes were carried out under the hood and rubber gloves and a lab coat were worn to prevent contamination.

Data Gathering

When hatching was completed the water was removed from the culture chambers and replaced with 10% buffered seawater-formalin. Formalin was flushed through the system several times to remove as much tritium as possible and the contents were then placed in glass vials for storage. To determine mortality and molting index the vials were shaken and approximately half the contents poured into a gridded 35 ml plastic petri dish. The dish was then placed under a clean microscope and the solution was allowed to settle for several minutes. The dishes were examined at 60X and the number of unhatched eggs, stage I, and stage II nauplii larvae were counted. After counting, the sample was returned to the vial and the operation repeated. Abnormalities were tabulated by taking a subsample from the unshaken vial with a pipette and preparing a wet mount slide for microscopic examination at 160X. The numbers of eggs, normal stage I, normal stage II, abnormal stage I, and abnormal stage II nauplii larvae were recorded.

An attempt was made during a preliminary study to examine larval chromosomes for obvious abnormalities. Colchicine was

introduced into the medium for periods varying between two hours and three days in order to arrest mitotic cell division but this was not successful. Several techniques were then used in attempts to isolate and spread chromosomes. The simplest method was to simply squash the larvae on a slide and try to stain the chromosomes. Another technique involved squashing and heating to rupture the nuclear membrane. In addition, some larvae were ground, treated with sodium citrate, centrifuged, and squashed with and without heat. Several stains were utilized and aniline blue in 45% lactic acid was the most promising. Unfortunately, at the 1600X limit of magnification, the chromosomes appeared to be mere pinpoints, so the chromosome portion of the study was abandoned after many fruitless attempts.

The preliminary studies established: that the eggs must be cultured in masses and not separated; that the water must be changed daily; and that lactic acid is a good preservative, although it was not used during the final study.

Liquid Scintillation Counting

At the completion of the experiment, samples of the water were taken and mixed with Scintisol Complete, a prepared cocktail available from New England Nuclear, in a 15:1 solvent-to-solute ratio. A standard was prepared using calibrated tritiated water in the same ratio.

A surprisingly high efficiency of 42% was obtained when the solutions were counted on a Packard Tri-Carb liquid scintillation counter. Excessive coincidence loss was encountered with the hottest sample but time did not allow a dilution to be made. The lowest activity sample was also discounted due to high background. Window settings of 50 to 850 were used with a gain of 80%.

III. RESULTS

Mortality

Egg viability (mortality) was defined as:

$$\frac{(\text{no. stage I nauplii larvae} + \text{no. stage II nauplii larvae})\,100}{\text{no. stage I nauplii larvae} + \text{no. stage II nauplii larvae} + \text{no. eggs}}$$

The data are summarized in Table I. There was no apparent pattern or relationship between HTO concentration in which the eggs were maintained, absorbed dose and egg viability.

Molting Index

The molting index, $\frac{(\text{no. stage II nauplii larvae})\,100}{\text{no. stage I nauplii larvae}}$, is a measure of the success that stage I larvae had in molting to stage II larvae. It is apparent that molting success was adversely affected at all levels tested when compared to the controls, as seen in Table II and Figure 4 and the effect was proportional to the absorbed dose. Rather surprisingly, the data points in Figure 4 resulted in a slightly curved line. The most striking part of Figure 4 is the control value, which could not be conveniently placed on the same graph. The control molting index of 42. 2 is over three times the 10^{-5} value. Since the high molting index of the controls could have come about as a result of a decrease in the number of stage I larvae or an increase in stage II larvae, it is interesting to look at the numbers in Table II.

Table I. Viability of P. polymerus eggs raised in various concentrations of HTO.

HTO concentration μCi/ml	$\frac{\text{(no. stage I nauplii larvae + no. stage II nauplii larvae) 100}}{\text{no. stage I nauplii larvae + no. stage II nauplii larvae + no. eggs}}$	viability
10^{1}	$\frac{(3160+129)100}{3160 + 129 + 2619}$	55.7%
10^{-1}	$\frac{(3658+294)100}{3658 + 294 + 1770}$	69.1%
10^{-3}	$\frac{(3894+424)100}{3894 + 424 + 4140}$	51.1%
10^{-5}	$\frac{(2507+318)100}{2507 + 318 + 1777}$	61.4%
0	$\frac{(3804+1606)100}{3804 + 1606 + 2572}$	67.8%

Table II. Molting indices and numbers of eggs, stage I, and stage II P. polymerus nauplii larvae raised in various concentrations of HTO.

concentration μCi/ml	count	no. eggs	no. stage I	no. stage II	total	molting index
0	1st	1171	1795	874	3840	48.69
0	2nd	1401	2009	732	4142	36.44
0	combined	2572	3804	1606	7982	42.22
10^{-5}	1st	1033	1450	186	2669	12.83
10^{-5}	2nd	744	1057	132	1933	12.49
10^{-5}	combined	1777	2507	318	4602	12.68
10^{-3}	1st	2256	2116	231	4603	10.92
10^{-3}	2nd	1884	1778	193	3855	10.85
10^{-3}	combined	4140	3894	424	8458	10.89
10^{-1}	1st	856	1699	132	2687	7.77
10^{-1}	2nd	914	1959	162	3035	8.27
10^{-1}	combined	1770	3658	294	5722	8.04
10^{1}	1st	1471	1769	61	3301	3.45
10^{1}	2nd	1148	1391	68	2607	4.89
10^{1}	combined	2619	3160	129	5908	4.08

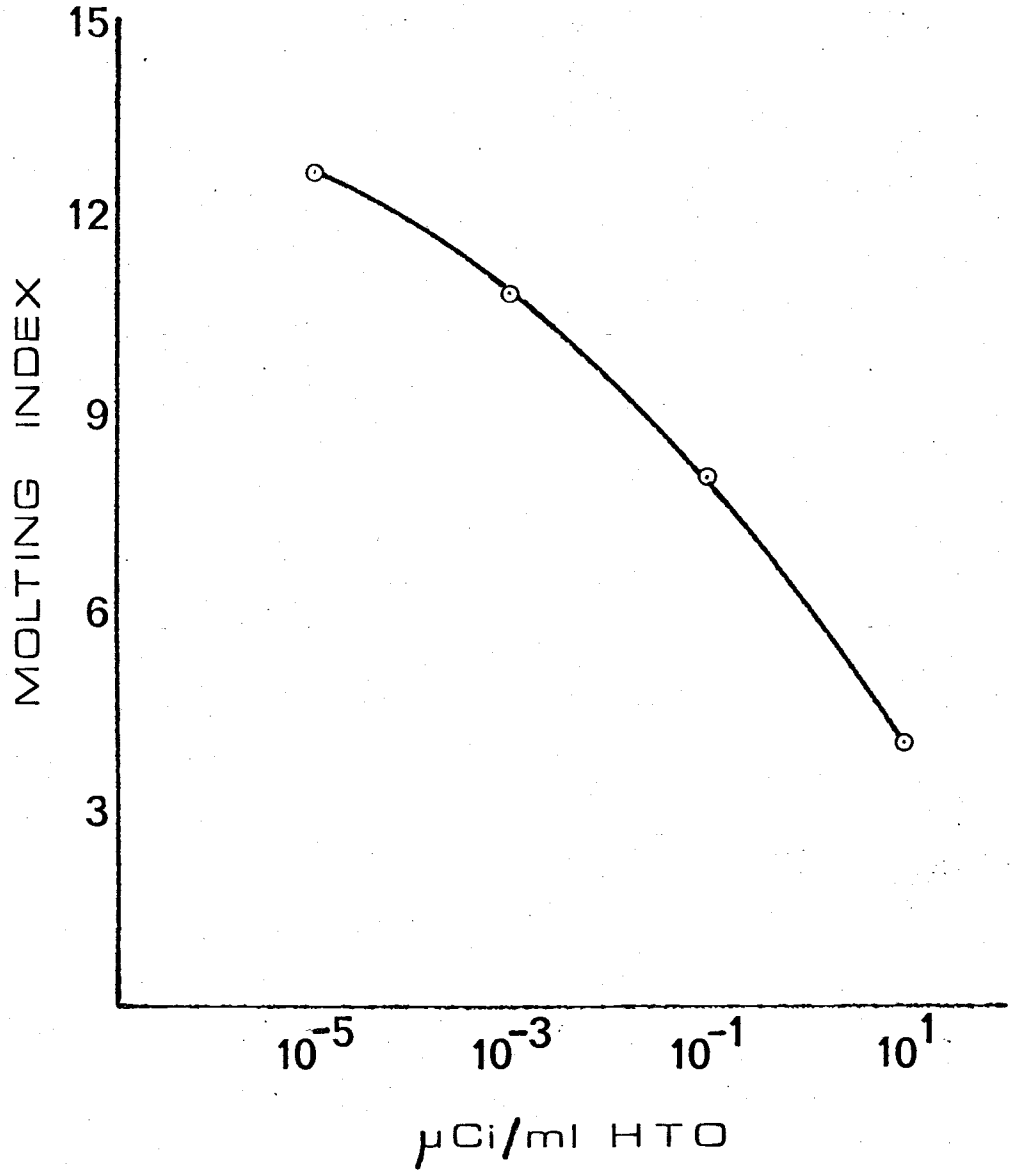

Figure 4. Molting indices of larvae raised in various concentrations of HTO. Control molting index = 42. 2.

There was clearly an increase in stage II larvae and not an unrelated decrease in stage I larvae which was responsible for the high control value. This indicates that a drop in mortality of stage II larvae occurred rather than an increase in stage I larvae and this is significant because if the incidence of abnormalities parallels mortality, it would be expected that there would be a larger number of stage II abnormalities than stage I at 10^{-5} μCi/ml, as will be seen. Typical eggs, stage I, and stage II larvae can be seen in Figures 5 and 6. A stage I larva molting to stage II larva can be seen in Figure 7.

Abnormalities

The percentages of larvae determined to be abnormal are listed in Table III, along with the numbers of animals examined. As with mortality and molting index data, two counts were conducted on each culture and the data pooled. The percentages in Table III are graphically displayed in Figure 8. It is significant that there was a higher incidence of abnormalities in stage II larvae at 10^{-5} μCi/ml than the controls. Not only does this confirm the finding that it was predominantly the stage II larvae which were affecting the molting indices at low concentrations but it also mathematically explains the slight shoulder seen at low concentrations in Figure 4. For stage II larvae the effect was proportional to the absorbed dose. This was also true for stage I larvae raised in 10^{1}, 10^{-1}, and 10^{-3} μCi/ml, but not in

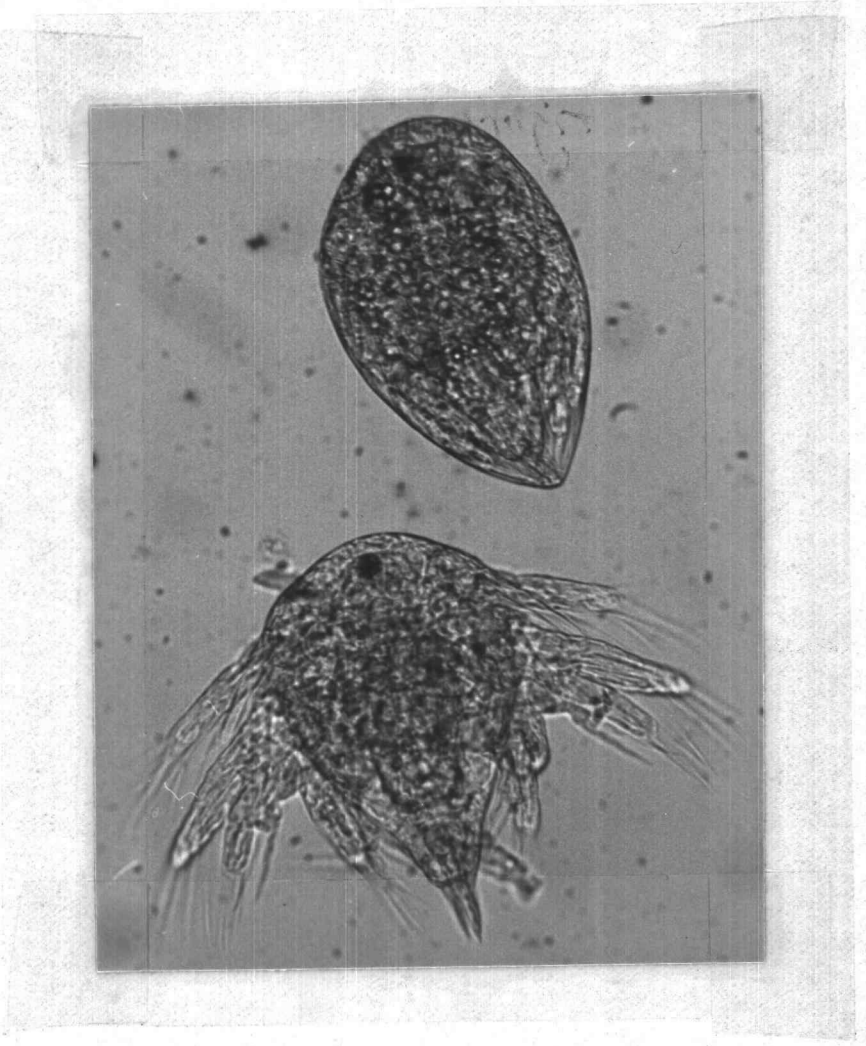

Figure 5. A normal unhatched egg and a stage I nauplius larva (bottom) of P. polymerus. Approximately 128X.

Figure 6. A normal stage I (left) and a normal stage II nauplius larva of P. polymerus. Approximately 128X.

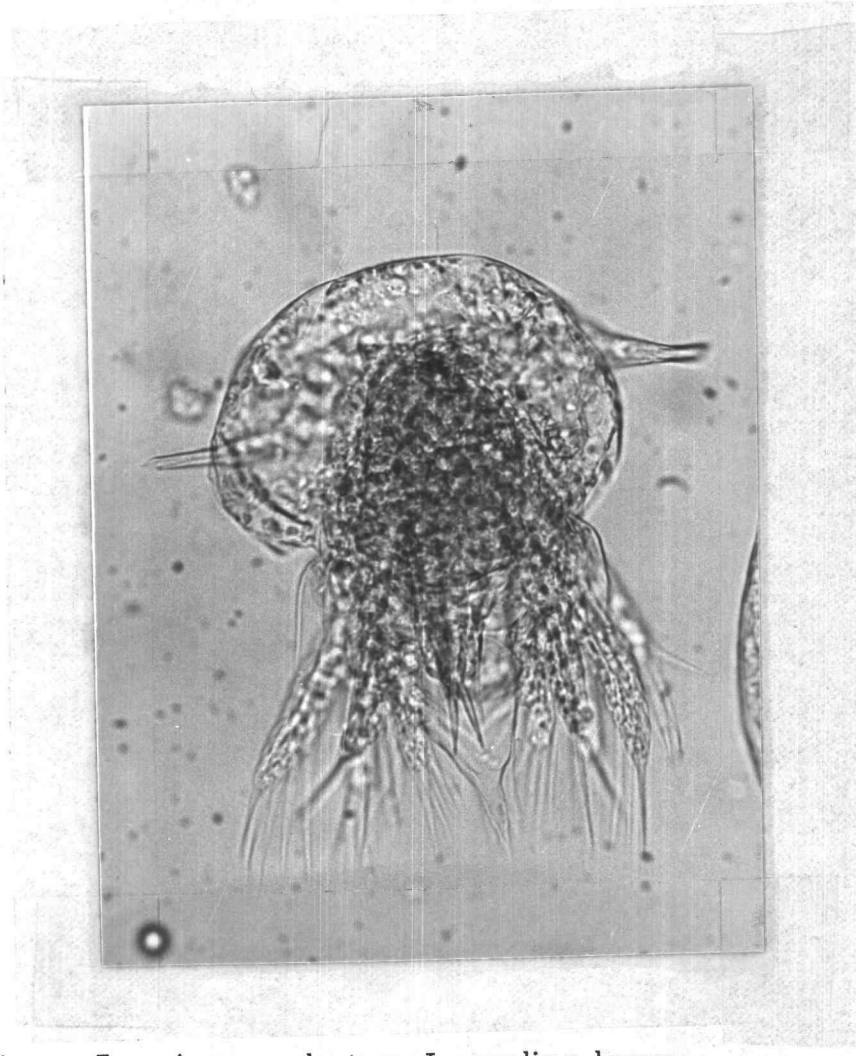

Figure 7. A normal stage I nauplius larva molting to stage II nauplius larva of P. polymerus. Approximately 128X.

Table III. Percentages of abnormal P. polymerus larvae and numbers of abnormal and normal stage I and stage II nauplii larvae raised in various concentrations of HTO.

HTO concentration μCi/ml	normal stage I	abnormal stage I	normal stage II	abnormal stage II	% stage I abnormal	% stage II abnormal
0	562	4	247	0	.71	0
0	194	1	97	0	.52	0
combined	756	5	344	0	.66	0
10^{-5}	118	0	34	0	0	0
10^{-5}	568	3	102	4	.53	3.92
combined	686	3	136	4	.44	2.94
10^{-3}	568	8	44	2	1.41	4.55
10^{-3}	293	2	20	3	.68	15
combined	861	10	64	5	1.16	7.81
10^{-1}	197	9	10	2	4.57	20
10^{-1}	671	31	23	1	4.62	4.35
combined	868	40	33	3	4.61	9.09
10^{1}	945	32	22	3	3.39	13.64
10^{1}	132	6	10	0	4.55	0
combined	1077	38	32	3	3.53	9.38

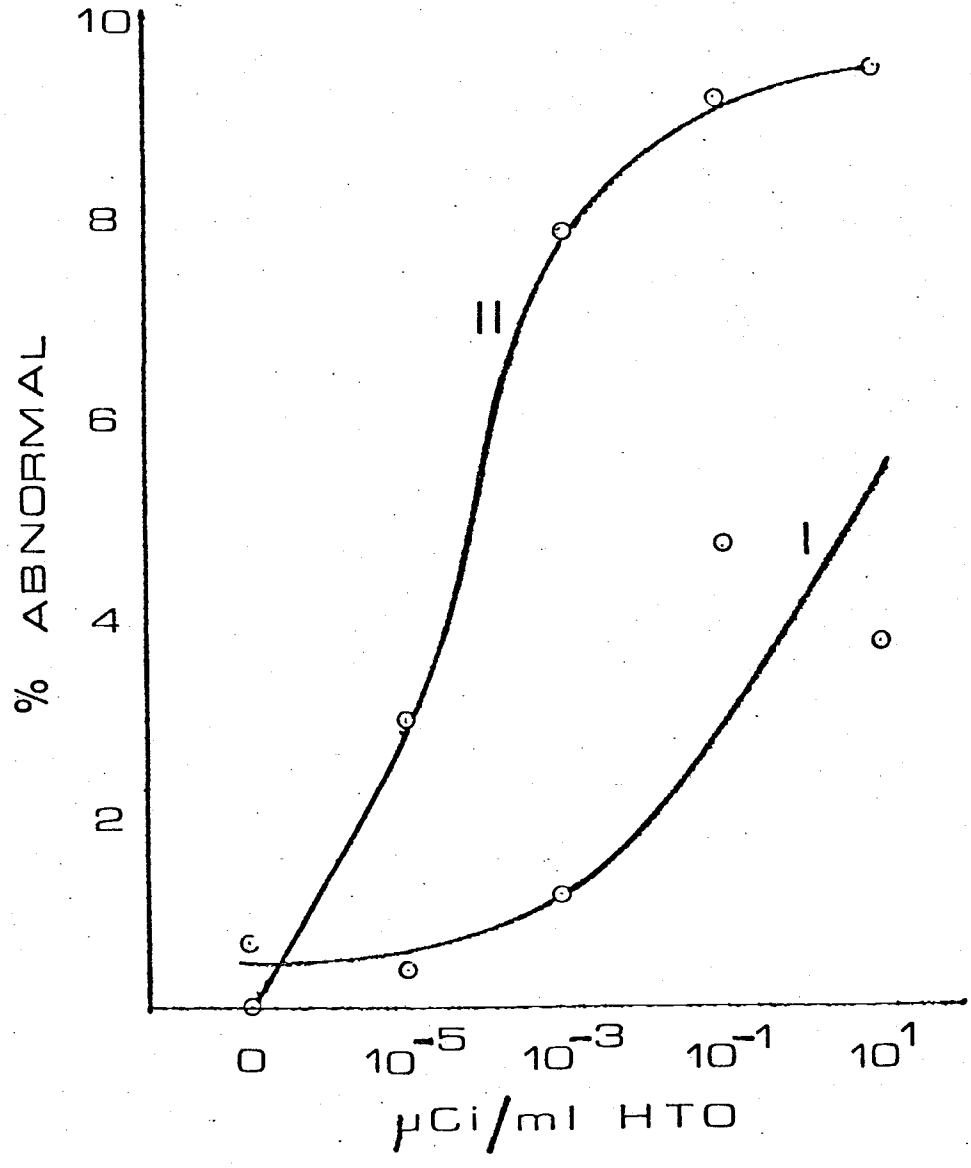

Figure 8. Percentages of abnormal stage I and stage II P. polymerus nauplii larvae raised in various concentrations of HTO.

10^{-5} μCi/ml. Stage I abnormality incidence appears to exhibit a threshold between 10^{-3} and 10^{-5} μCi/ml below which no effect can be demonstrated. Numbers of specific types and locations of abnormalities are summarized in Table IV. It is evident that stage I larvae generally had abnormalities at the anterior end while stage II larvae showed more posterior abnormalities. Typical abnormalities appear in Figures 9 through 16.

Liquid Scintillation Counting

Table V contains the results of the liquid scintillation counting and the calculated absorbed doses (Shapiro, 1972); the assumption was made that the activity of the eggs was the same as the activity of the water. The counted concentration for the 10^{1} sample was low due to high coincidence loss. Time did not allow for correction of this deficiency. The formula used for calculating the absorbed dose was 51 CE_{β^-}-rads/day times the number of days exposure where C = concentration in μCi/gm and E_{β^-} = average β^- energy per disintegration in MeV. The lowest concentration counted is also questionable because of the high background. The results indicate there was good agreement between calculated and actual tritium concentrations.

Table IV. Numbers of specific abnormalities and a summary of body regions affected (at bottom) in P. polymerus nauplii larvae.

Abnormality	10^{-5}		10^{-3}		10^{-1}		10^{1}		0	
	I	II	I	II	I	II	I	II	I	II
fish head	x	x	1	x	x	x	7	x	x	x
mushroom head	x	x	1	x	32	x	16	x	x	x
fireplug head	x	x	3	x	3	x	5	x	x	x
gross anterior	1	1	2	x	x	x	1	x	1	x
squid	x	x	x	x	1	x	x	x	x	x
balloon	x	x	x	x	1	2	x	x	x	x
crabhead	x	x	x	1	x	x	x	x	x	x
lumpy	x	x	x	x	x	x	2	x	x	x
dorsal protrusion	x	x	x	x	x	x	1	x	x	x
unilateral protrusion	x	x	1	x	x	x	1	x	3	x
appendage loss	x	x	1	x	x	x	x	x	x	x
ventral	2	x	x	x	x	x	x	x	x	x
thin midsection	x	x	x	x	1	x	1	1	x	x
short tail	x	3	x	4	x	1	x	3	x	x
gross posterior	x	x	1	x	x	x	x	x	x	x
Daphnia like	x	x	x	x	x	x	4	x	x	x
lumpy	x	x	x	x	x	x	2	x	x	x
gross	x	x	x	x	2	x	x	x	1	x
gross general	x	x	x	x	2	x	4	x	1	x
anterior	1	1	7	1	37	2	29	x	1	x
middle	2	x	2	x	1	x	5	1	3	x
posterior	x	3	1	4	x	1	x	3	x	x

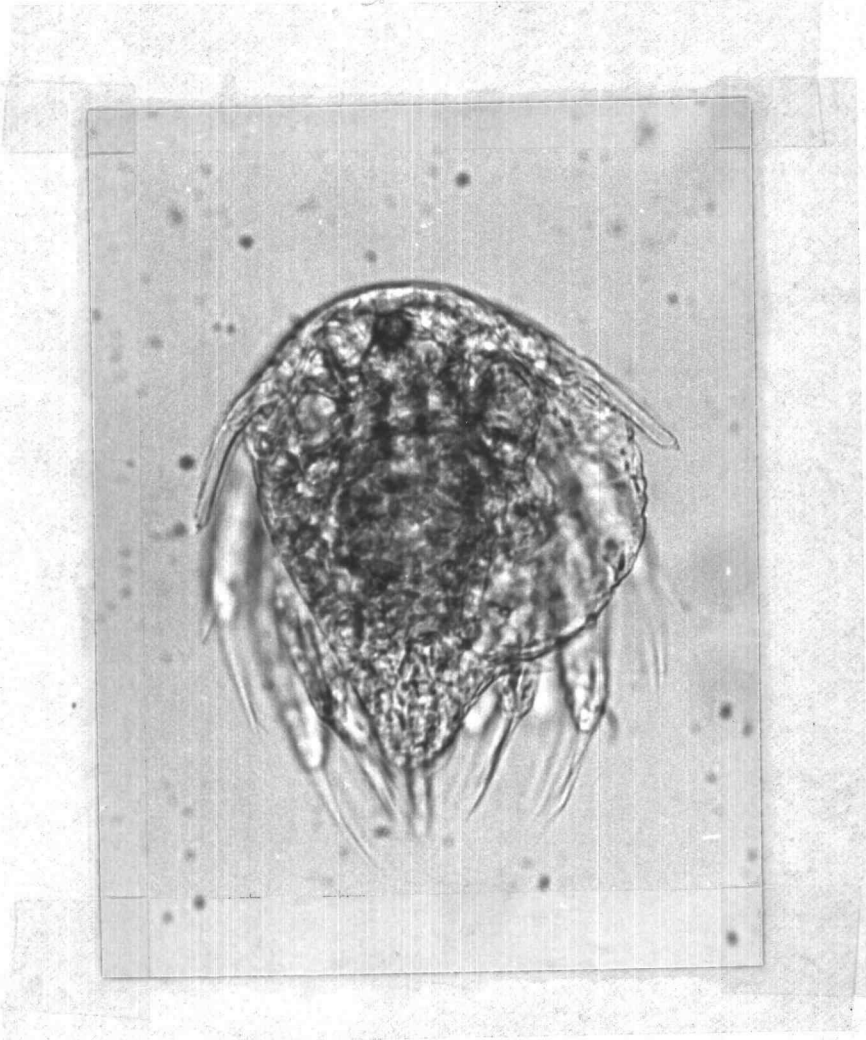

Figure 9. An abnormal stage I P. polymerus nauplius larva raised in 10^1 μCi/ml HTO with a "unilateral protrusion." Approximately 128X.

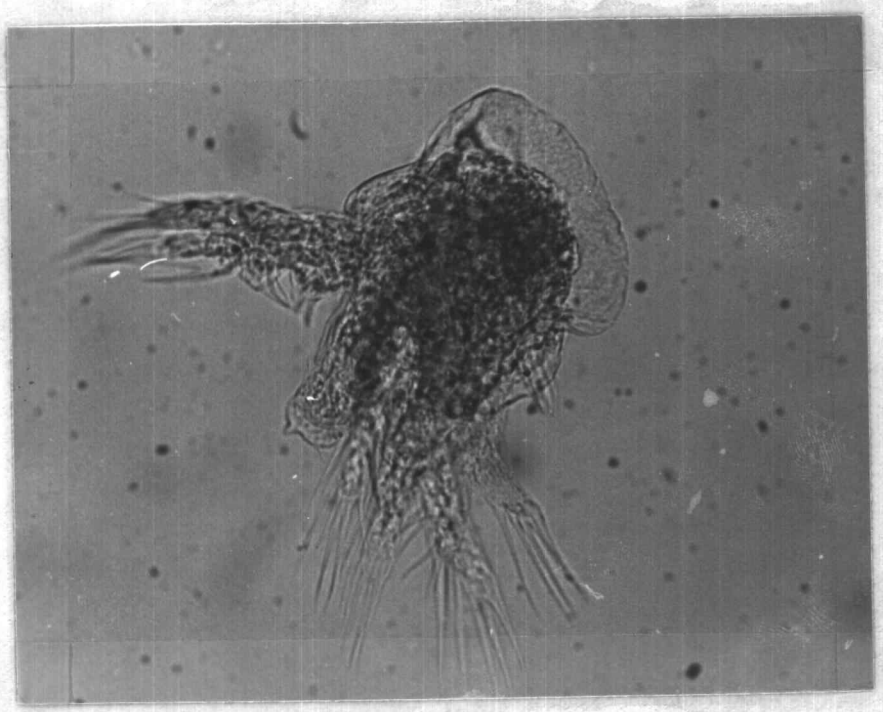

Figure 10. An abnormal stage I P. polymerus nauplius larva raised in 10^{-1} μCi/ml HTO with a "mushroom head." Approximately 200X.

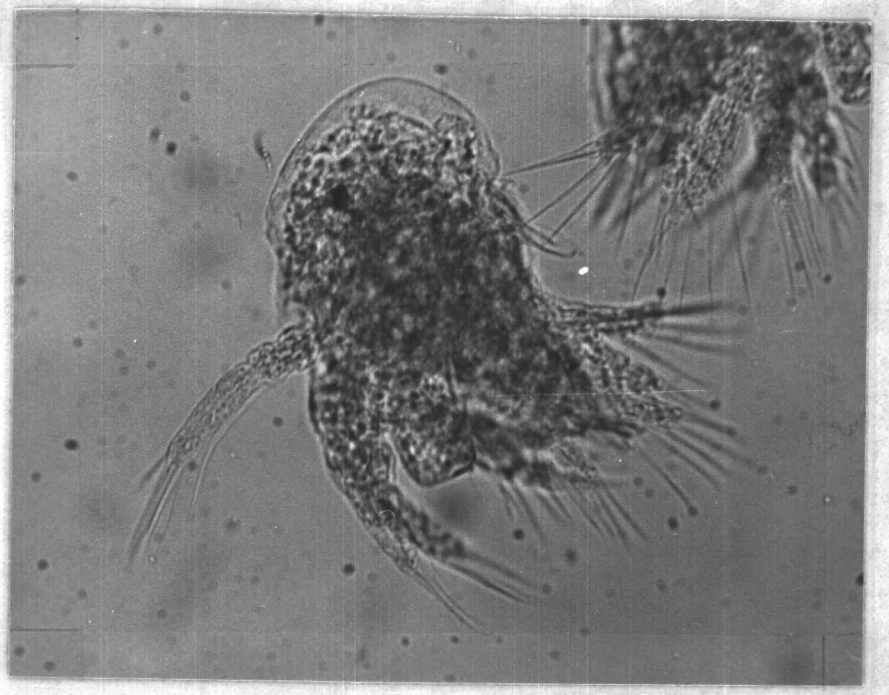

Figure 11. An abnormal stage I P. polymerus nauplius larva raised in 10^{-1} μCi/ml HTO with a "balloon head." Approximately 150X.

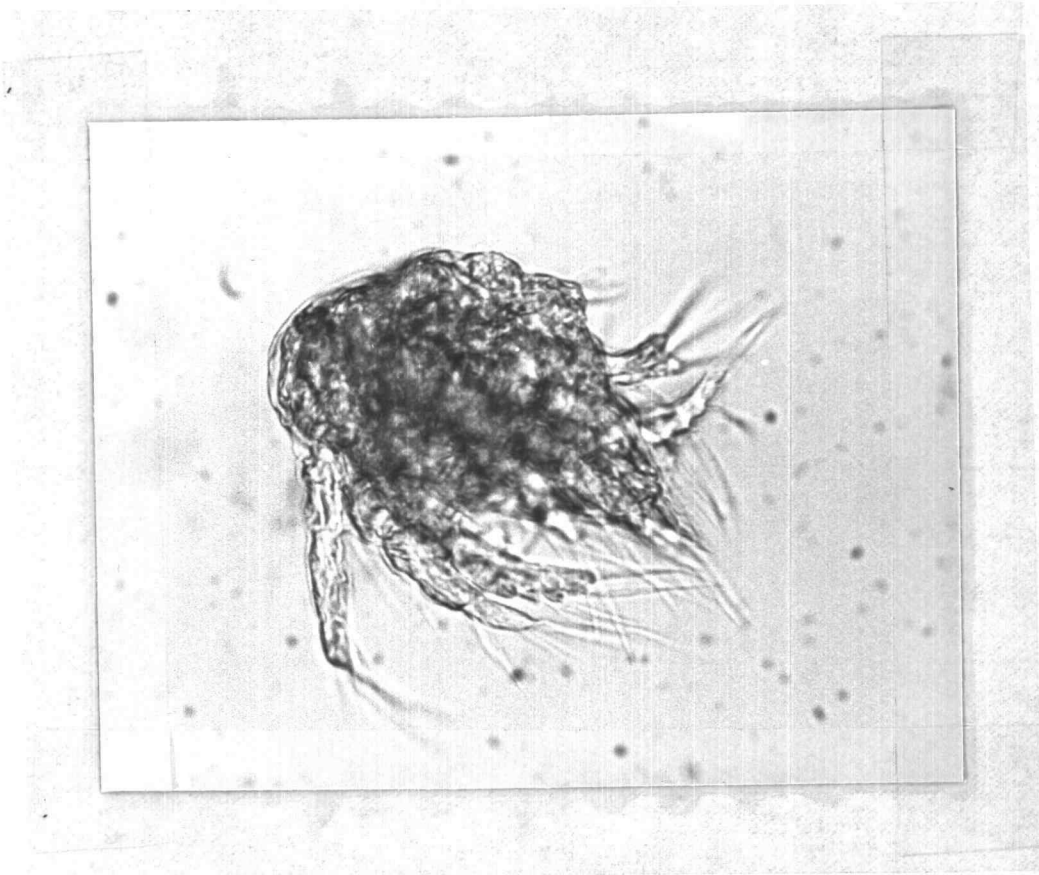

Figure 12. An abnormal stage I P. polymerus nauplius larva raised in 10^{1} μCi/ml HTO designated "lumpy." Approximately 128X.

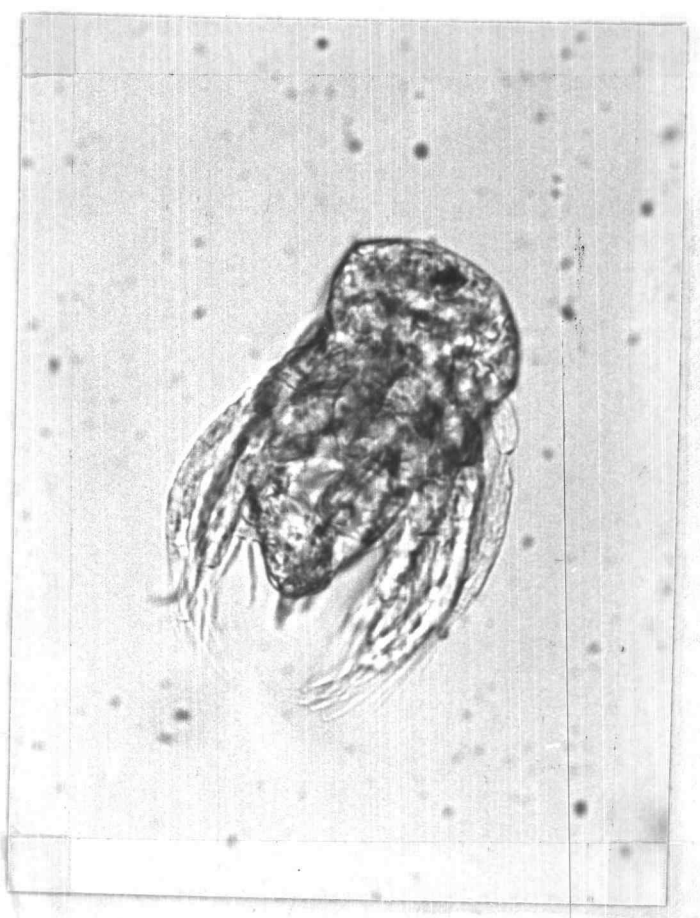

Figure 13. An abnormal stage I P. polymerus nauplius larva raised in 10^{1} µCi/ml HTO with a "fireplug head." Approximately 200X.

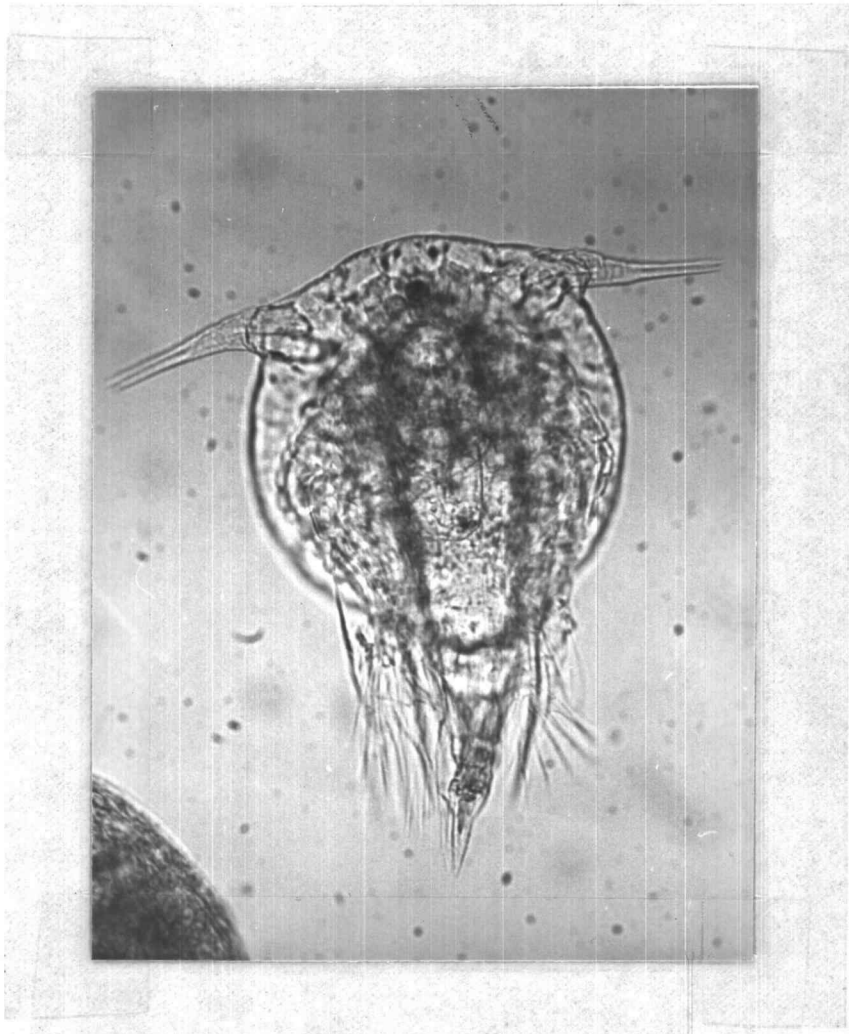

Figure 14. An abnormal stage II P. polymerus larva raised in 10^{-3} μCi/ml HTO with a deformed tail. Approximately 150X.

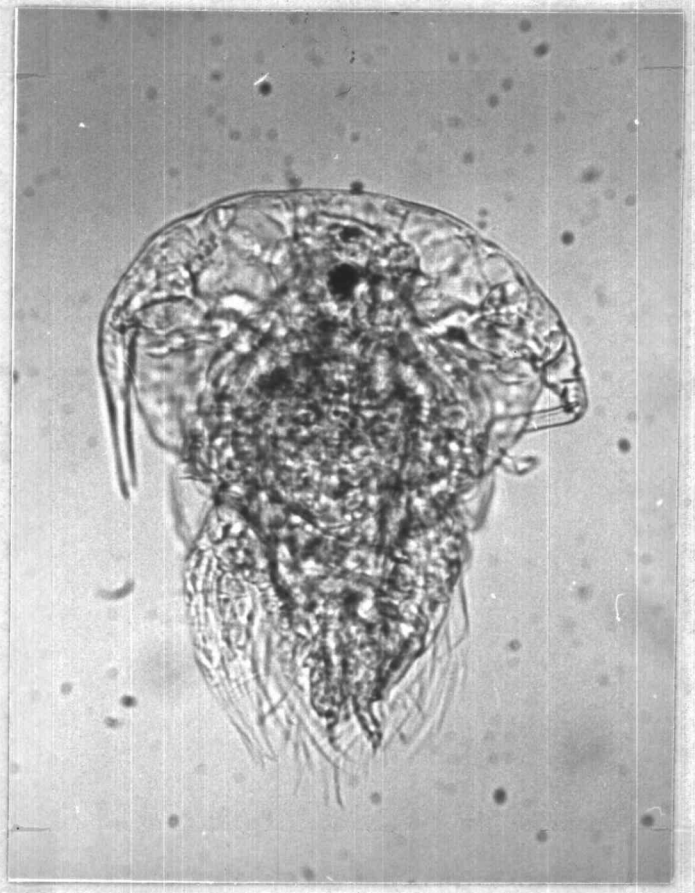

Figure 15. An abnormal stage II P. polymerus nauplius larva raised in 10^{-3} μCi/ml HTO with a "crab head." Approximately 150X.

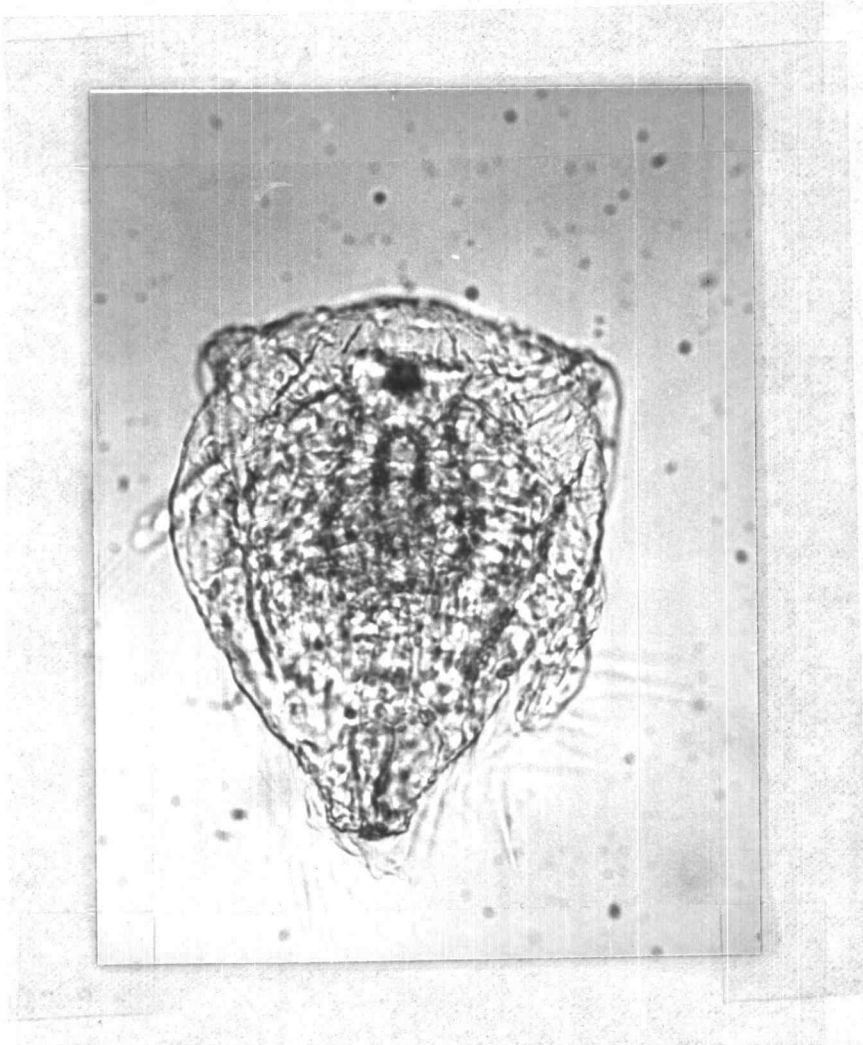

Figure 16. An abnormal stage II P. polymerus nauplius larva raised in 10^{-3} μCi/ml HTO with no tail. Approximately 150X.

Table V. HTO concentrations determined by liquid scintillation counting and total absorbed dose in the various concentrations of HTO.

Stated concentration	Calculated concentration	Total absorbed dose
10^{1} μCi/ml	3×10^{0} * μCi/ml	67 rads
10^{-1} μCi/ml	8.3×10^{-2} μCi/ml	673 mrads
10^{-3} μCi/ml	1.16×10^{-3} μCi/ml	6.73 mrads
10^{-5} μCi/ml	3.25×10^{-4} μCi/ml	6.7×10^{-2} mrads

*Uncorrected for high coincidence loss

IV. DISCUSSION

The results of this study indicate that developmental stages of P. polymerus are quite radiosensitive. Various theories have been proposed to account for the relative radiosensitivity of organisms. One of the most popular is that of Sparrow (1963), which states that chromosome volume is directly proportional to radiosensitivity; i. e., as the size or volume of chromosomes increases, so does radiosensitivity. For this reason attempts were made to evaluate the effects of HTO on P. polymerus chromosomes. Unfortunately that portion of the experiment was abandoned because of the very small size of the chromosomes. However, it seems unlikely that Sparrow's hypothesis would be of aid in explaining the extreme radiosensitivity found in Pollicipes polymerus. An alternative hypothesis (Patt and Quastler, 1963) is that the level of metabolic activity or division rate of tissue is directly proportional to the radiosensitivity. This hypothesis is partially supported by the abnormality data, where most aberrations appeared in regions of actively dividing tissue. While this is adequate for explaining the location of abnormalities, it does not necessarily account for the radiosensitivity of this species.

It seems likely that the extreme radiosensitivity of Pollicipes may be related to its pattern of development. This barnacle undergoes determinate or mosaic development and the fate of individual

cells is determined or fixed as early as the fourth division (Anderson, 1973). The unequal holoblastic, spiral, determinate development is prolonged and approximately one month is required for the zygotes to develop to the hatching stage. Another month is required before the larvae metamorphose into adults. This prolonged determinate development allows sufficient time for uptake and equilibrium to occur as well as increasing the amount of time available for fatal damage to occur in the cells. In determinate cleavage, the loss of a single cell can result in the loss of a large portion of the animal if it occurs early in development. Generally, the later a cell is killed the smaller the area that will be affected. Since in this study, development of the embryos was about two thirds completed by the beginning of the experiment, the sensitivity may be even greater for earlier embryos. In all probability a significant effect on hatchability would be seen if exposure began at earlier stages.

The theory that low levels of HTO may cause negative biological effects is supported by evaluating the effect of HTO in the molting index. Often the effects of radiation are delayed and show up later in development and, indeed, it appears to have occurred in this study. No clear pattern can be seen in the viability of embryos when hatching was the criterion used but this was not totally unexpected, since development was advanced at commencement of exposure. However, when the success at molting (a very important aspect of crustacean

life) was determined, it was clear that the HTO had caused an adverse effect. Thus a subtle negative biological effect was observed which would have been missed if only hatching data were considered.

The matter of direct versus indirect action of HTO can not be resolved by this experiment. Direct action requires the damage to be at the same location as the observed effect, while indirect action stipulates that the damage must be distant from the location of the observed effect. The abnormalities would seem to indicate that death occurred directly in cells where the anomaly was seen, since the damage appeared in the region of active growth and no known mechanism of indirect action is apparent. However, the effect on molting could be indirect. It is not difficult to conceive of damage to an organ whose metabolites would ultimately affect the secretion of ecdysone, the molting hormone. It seems likely that both modes of damage were involved.

Several characteristics of tritium may contribute to its somewhat surprising ability to cause biological damage. Ueno (1974) demonstrated that tritium is incorporated into DNA and RNA from HTO during the synthesis of these vital substances in fish eggs. Lea (1955) calculated the optimum specific ionization needed for beta particles to produce damage to DNA, and this value is surprisingly close to that of tritium. The linear energy transfer (LET) of beta particles rises sharply as the speed (energy) decreases at low levels

and thus, tritium's average beta energy of 5.7 keV gives it a higher LET than any other commonly encountered beta emitter. This high LET with its associated high specific ionization may be the reason for tritium's suspected effectiveness in damaging DNA. Since some of the tritium will be incorporated into the genetic material of organisms developing in an HTO environment, the possibility exists that there may be long term effects to both the individual animal and the entire population.

The results of this study may have serious implications. If the increased mortality of larvae resulted in a decrease in the numbers of adults, the stability of the Mytilus-Pollicipes-Pisaster community could be upset. The decrease in the number of larvae could have an effect on those organisms dependent on small-sized organisms for food, although this is not too likely. Of greater importance is the question of radiosensitivity of other crustacean nauplii larvae. If other crustacean species have similar radiosensitivity, such populations may be adversely affected if HTO levels approach current standards. The current maximum permissible concentration in water is 3×10^{-3} μCi/ml, a level clearly detrimental to goose barnacles. Re-examination of this standard may be in order if future studies on other invertebrates show similar results.

V. SUMMARY

The objective of this study was to determine the biological effects of HTO on developing goose barnacle embryos and larvae. Pollicipes polymerus was considered to be appropriate for study because: it is a common marine invertebrate, a largely neglected group in HTO effects studies; it has a prolonged developmental period, thereby insuring equilibration, uptake and incorporation of tritium; it is an ecological dominant in the Mytilus-Pollicipes-Pisaster community; methods for culturing its eggs have been developed; and its eggs are readily available. Egg masses were cultured in HTO concentrations of 0, 10^{1}, 10^{-1}, 10^{-3}, and 10^{-5} μCi/ml until hatching was completed after 22 days, at which time they were fixed in formalin.

Several criteria were used to evaluate the biological effects of HTO on development. The traditional viability of eggs was examined, with no apparent effects noted. Attempts were made to examine chromosomes for visible aberrations, but technical difficulties and the small size of the chromosomes prevented success. The "molting index," a measure of the success with which stage I nauplii larvae have in molting to stage II, was utilized. Significant detrimental effects were observed at all HTO concentrations tested, with the effect being proportional to the absorbed dose. Finally, morphological abnormalities were counted and the percentages of abnormal

larvae were calculated for both stage I and II nauplii larvae. Stage II nauplii larvae were detrimentally affected at all HTO concentrations tested, while stage I nauplii larvae were affected at 10^{1}, 10^{-1}, and 10^{-3} μCi/ml, but not at 10^{-5} μCi/ml, thereby showing a threshold between 10^{-3} and 10^{-5} μCi/ml HTO. Stage I nauplii larvae consistently showed an anterior bias in the location of their abnormalities while stage II nauplii larvae were affected predominantly in the post-nauplier, or posterior, end.

Several hypotheses are available to explain the extreme radiosensitivity of Pollicipes polymerus. The explanation that a large chromosome volume was responsible is not likely due to the small size of the chromosomes. The idea that actively dividing or metabolically active tissues are very radiosensitive is adequate to explain the location of abnormalities but does not explain the overall radiosensitivity in view of the slow development of embryos and larvae. The prolonged development coupled with the determinate or mosaic pattern of development may explain the radiosensitivity of this species' developmental stages. The determinate cleavage of P. polymerus requires the loss of only a single cell to cause death or morphological abnormality. The prolonged development assures equilibration with the environment and incorporation of tritium in organic molecules as well as increasing the probability of fatal damage to cells. The long term genetic consequences to the

individual and the population remain unknown. If the observed radiosensitivity of goose barnacle larvae proves to be indicative of general marine crustacean radiosensitivity, a re-examination of the current MPC_w (3×10^{-3} μCi/ml) for tritium may be in order.

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