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TRITIUM UPTAKE KINETICS IN CRAYFISH
(*Orconectes immunis*)

Report No 85-183-K

P.H. Patrick
Research Biologist
Biological Research Section
Chemical Research Department

RESEARCH

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ABSTRACT

Uptake of tritiated water (HTO) by *Orconectes immunis* was investigated under laboratory conditions. Tritium uptake in the tissue-free water fraction (TFWT) was described using an exponential model. When steady-state was reached, the ratio of TFWT to HTO was approximately 0.9. Uptake of tritium in the organically-bound fraction (OBT) proceeded slowly, and had not reached steady-state after 117 days of culture. Although steady-state was never reached, the maximum observed ratio of OBT to TFWT in whole animals was approximately 0.6. However, this ratio exceeded unity in the exoskeleton. Specific activity ratios of OBT between crayfish and lettuce (food source) were less than or at unity for various test conditions.

job	file	date	report no.
740634-572-490	837.88	June 14, 1985	85-183-K

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EXECUTIVE SUMMARY

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In an effort to obtain a better understanding of the fate and behaviour of tritium in the aquatic environment, a multi-phase biological research program has been undertaken. This study deals with phase II (primary consumer) of the program, and investigates the kinetics of tritium uptake in crayfish (*Orconectes immunis*).

Tritium uptake in the tissue-free water fraction (TFWT) was described using an exponential model. In animals exposed to tritiated water or combined tritiated water and food, uptake rate constants were 19.5±5.8 (95% C.I.) day^{-1} and 22.1±5.7 day^{-1}, respectively. After 4 h exposure, the ratio of TFWT to HTO was approximately 0.9. Uptake of tritium in the organically-bound fraction (OBT) proceeded slowly, and had not reached steady-state after 117 days of culture. Highest OBT concentrations were observed in animals exposed to both tritiated food and water. Although steady-state was never reached, the maximum observed ratio of OBT to TFWT in whole animals was approximately 0.6. However, this ratio exceeded unity in the exoskeleton. The exoskeleton of *O. immunis* showed significantly lower TFWT, but higher OBT concentrations than muscle and visceral tissues. Specific activity ratios of OBT between crayfish and lettuce (food source) were less than or at unity for various test conditions.

These results provide further support for the common assumption held by the tritium specific model with the exception of the OBT/TFWT exoskeleton results which may be a function of metabolic isotopic discrimination of tritium versus hydrogen.

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To Mr. F.J. Kee
Director of Research

TRITIUM UPTAKE KINETICS IN CRAYFISH (*Orconectes immunis*)

INTRODUCTION

Although tritium is an important release product of nuclear generating stations, especially those using CANDU reactors, its fate and behaviour in the aquatic environment are not well understood. For instance, it still has not been firmly established whether tritium accumulates or concentrates in various trophic levels of a food chain. Although most laboratory investigations have indicated little or no evidence of bioaccumulation within or between trophic levels (Bonoto et al, 1977; Komatsu et al, 1981), some field data does suggest the possibility of bioaccumulation (Kirchman and Dupont, 1980).

In an effort to obtain a better understanding of the fate and behaviour of tritium in the aquatic environment, a multi-phase biological research program has been undertaken. A key objective of the program is to determine the specific activity relationships between primary producers and consumers of typical aquatic food chains. This study deals with Phase II (primary consumer) of the program and investigates the kinetics of tritium uptake in crayfish. Animals were exposed to tritium when tritium was ingested from the food, absorbed from the medium, and ingested both from the food and the medium.

MATERIALS AND METHODS

Orconectes immunis, a common crayfish of Ontario (Crocker and Barr, 1971), was used as the test organism. During late June and early July, approximately 1800 individuals (mean wt 0.75 g wet, mean length 15.0 mm metasome) were collected from the Selkirk Creek drainage area located near Nanticoke GS on Lake Erie. Previous sampling at this location indicated that the population was univoltine, with eggs hatching from mid-May to mid-June.

2. Experimental Holding Tanks

Approximately 500 crayfish were held in large (250 cm (l) x 250 cm (w) x 38 cm (h)) polyethylene tanks, each containing approximately 710 L of dechlorinated water (Figure 1). Surface area of each tank was 6.3 m^2. A closed flow-through water recycle system was used with a flow rate of ∿66 Lpm. Each tank had a biofilter (45 cm x 55 cm x 40 cm) composed of gravel and cobble material, which appeared to be effective in filtering out the particulate wastes. Crayfish were held under natural photoperiod (12 to 14 h light) over the July to November experimental period. All tanks were covered with a clear lexan plastic. A number of tubes (PVC pipe 10 cm x 5 cm) were placed horizontally in each tank to provide cover for crayfish. Tank water temperatures showed a general decrease over the testing period. Mean temperature generally ranged from 19 to 22°C where optimal growth in crayfish was expected to occur (Winberg, 1971). Higher temperatures, exceeding 23°C, were observed in the initial few weeks of monitoring (Appendix A), when animals were noted to be sluggish and did not feed well. After installing cooling coils, temperatures generally did not exceed 22°C over the testing period ($\bar{x}$ = 20.5±1.5°C).

3. *Lettuce Food Source*

Lettuce (*Lactuca sativa* L cv Grand Rapids) was grown as the sole crayfish food source. Since *Orconectes immunis* is largely herbivorous, feeding on aquatic vegetation and terrestrial plants (Tack, 1941; Crocker and Barr, 1971), lettuce was considered an acceptable food source. Other advantages of using lettuce as a food source are that it can be easily labelled with tritium (HTO) and can be grown in sufficient quantities in a relatively short time period (6-10 weeks). Lettuce seeds were germinated in flats, seedlings were transplanted into plastic pots containing Redi-Earth (Grace & Co), and grown in a tritiated environment (irrigation and atmosphere) in a commercially manufactured growth chamber (Conviron Model E15). A detailed description of the growth chamber is presented in Ontario Hydro Research Division Report No 83-33-K.

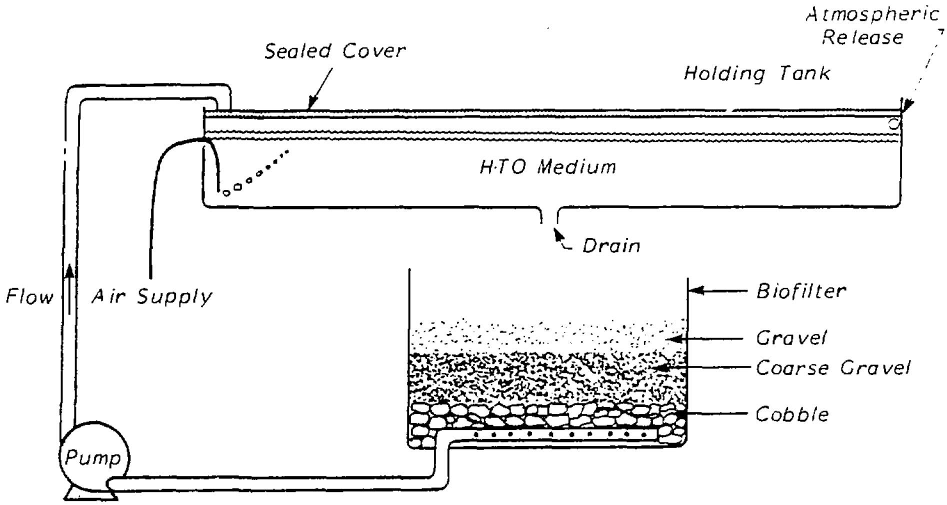

FIGURE 1

SIDE VIEW SHOWING EXPERIMENTAL HOLDING TANK AND SECTION OF BIOFILTER

Lettuce seedlings were exposed to a mean atmospheric concentration of 23.3 µCi L^{-1} tritiated water vapour, and 49.0 µCi L^{-1} irrigation water (Ontario Hydro Research Division Report No C82-125-K). Air temperature was 18°C at night and 23°C during the day (relative humidity 70% day, 60% night). Light intensities, measured with a quantum light meter (Lambda Model L1-195), ranged from 200-300 $\mu E \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density (PPFD) at the canopy level. Two harvests totalled approximately 4.8 kg of tritiated lettuce foliage. Mean tritium concentration as tissue-free water tritium (TFWT) and organically-bound tritium (OBT) fractions were as follows:

	Tritium Concentration (based on 36 samples)			
	TFWT (p Ci mL^{-1} of water)		OBT (pCi g^{-1} dry wt)	
Harvest	$\bar{x}$	S.D. ($\bar{x}$)	$\bar{x}$	S.D. ($\bar{x}$)
1	38 503	3 981	8 977	94
2	37 421	1 227	9 747	150
Overall Mean	37 962		9 363	

Tritium concentrations with respect to plant parts (upper and lower leaf portions), and location in the growth chamber are given elsewhere (Ontario Hydro Research Division Report No C82-125-K). Tritiated lettuce samples were composited and stored at -20°C. Approximately 2.0 kg of unlabelled lettuce was also grown, frozen and stored.

4. Experimental Procedure

Uptake kinetics of tritium in *Orconectes immunis* as TFWT and OBT fractions were determined when tritium was absorbed from the medium, ingested in organic form (lettuce), and both these sources combined (absorbed from the medium and ingested in organic form). Approximately 500 individuals (mean wt 0.75 g wet, mean length 15.0 mm metasome) were placed into separate holding tanks for each test criterion.

Because of an increase in mortality due to handling (mechanical/stress related), crayfish were not transferred into a separate feeding area after day 17 of testing. After a feeding, all lettuce and particulate wastes in each experimental tank were removed by siphoning, and discarded (to reduce potential contamination errors).

Duration of the experiment was 117 days (July 12 to November 5). For each experimental condition tritium uptake (as both TFWT and OBT) in crayfish was determined over the following time periods: 0, 1 (0.5, 1.0, 2.0, 4.0, 8.0, 10.0, 14.0, 18.0, 20.0, 24.0 h), 2, 4, 7, 10, 15, 18, 23, 30, 37, 51, 58, 88, 101 and 117 days.

Only one sample was collected from each time period, except at 0.5 h, 24.0 h, 10 days and 88 days of culture when replicates (n=3) were taken.

Each sample consisted of 10-15 individuals (8-22 g wt total). Analysis for tritium was done primarily on whole animals. Separate determinations of exoskeleton, and muscle and visceral tissues were attempted at the end of the sampling period (117 days). Crayfish which had died over the June to August experimental period were partitioned similarly and analyzed. Crayfish collected for tritium analysis were also weighed and measured (metasomal length) for growth ratio determinations. Crayfish were not randomly sampled, but biased in collecting the larger individuals because of limited "biomass" for analysis.

Tritium was introduced into two experimental tanks as tritiated water (HTO) to obtain a specific activity of ~40 mCi L^{-1}. The source solutions were prepared from 1.0 Ci mL^{-1} HTO purchased from Amersham Corp. Tritium concentration in the medium was monitored weekly and averaged approximately 34.0 mCi L^{-1} (1.82 S.D. ($\bar{x}$)) in the tritiated water and food test tank, and 38.3 m Ci L^{-1} (2.10 S.D. ($\bar{x}$)) in the tritiated water alone (unlabelled food) test tank. Tritium levels in the labelled food test tank (HTO not introduced) averaged 0.067 mCi L^{-1} (0.07 S.D. ($\bar{x}$)) over the monitoring period (less than 1% of the tritiated water test tanks).

5. Sample Preparation and Counting Procedure

Tissue-free water tritium (TFWT) in crayfish (visceral tissues, exoskeleton, whole animals) was extracted using azeotrophic toluene distillation (Mognissi et al, 1973; Ontario Hydro Research Division Report No 83-33-K). Each crayfish sample (8-22 g wet wt) provided 5-10 mL of tissue-free water. Recovered water was mixed with PCS II Amersham liquid scintillation cocktail (1 mL:14 mL) and tritium was counted in a Packard Tricarb-300C liquid scintillation counter.

Remaining residues following distillation were analyzed for organically-bound tritium (OBT). Samples were dried in a tritium-free fume hood overnight, then placed in a dessicator under vacuum over phosphorous pentoxide until all water was removed (as determined by constant weight measurement). Samples (1-2 g dry wt) were pressed into pellets (PAR Pellet Press), combusted in a Packard 360 Tri-Carb oxidizer, mixed with 18 mL Monophase-40 liquid scintillation cocktail, and then counted for tritium in a Packard Tri-carb 300C liquid scintillation counter.

Total organically-bound tritium in each sample was calculated. The contributions of exchangeable and non-exchangeable tritium components were not known. However, it was expected that the non-exchangeable component would be unchanged by sample processing, whereas the exchangeable component could result in either gains or losses of tritium content. Independent tests have indicated that low-level samples are not contaminated with high level samples during processing (Ontario Hydro Research Division Report No C83-37-K). For both TFWT and OBT analysis, control (crayfish material grown in a non-tritiated environment) determinations were made.

Tritium concentrations were expressed in both µCi L^{-1} of water and µCi g^{-1} H for TFWT, and in µCi g^{-1} H for OBT. Total hydrogen content of crayfish and lettuce was determined using a standard combustion gravimetry procedure (Analytical Research Services). Mean hydrogen content of whole crayfish ($\underline{n}$ = 3) was 47.8 g kg^{-1} (2.79 S.D. ($\bar{x}$)). Total mean hydrogen estimated in exoskeleton ($\underline{n}$ = 3), and muscle and viscera ($\underline{n}$ = 3) was 29.7 g kg^{-1} (1.53 S.D. ($\bar{x}$)), and 54.7 g kg^{-1} (0.6 S.D. ($\bar{x}$)), respectively. The estimates are similar to those reported by Dawirs (1980) for two decapod species. Mean hydrogen content of lettuce ($\underline{n}$ = 3) was 51.6 g kg^{-1} (1.15 S.D. ($\bar{x}$)).

6. Data Analysis

The uptake kinetics of tritium in crayfish was found to follow an exponential model:

$$[C_t = C_\infty (1 - e^{-kt})]$$

where C_t = tritium concentration at time t, expressed in µCi L^{-1}

C_∞ = steady-state concentration (asymptotic value) reached when dc/dt = 0

t = time in hours

k = initial uptake rate constant at t = 0, expressed in $days^{-1}$.

This analysis was done by Operations Research Services. Initial uptake rate constants (k), and asymptotic values were compared between animals exposed to tritiated water, and tritiated water and food test conditions using parametric statistics (Steele and Torrie, 1960).

RESULTS AND DISCUSSION

1. Uptake Kinetics of Tritium as Tissue-Free Water

Tritium uptake in the tissue-free water fraction (TFWT) was determined for animals exposed to tritiated water, and tritiated water and food. Uptake of tritium in both experimental conditions proceeded rapidly, presumably reaching steady-state after 4 h of exposure (Figure 2). The further increase in uptake after 40 days in the tritiated water tests probably reflect errors in methodology.

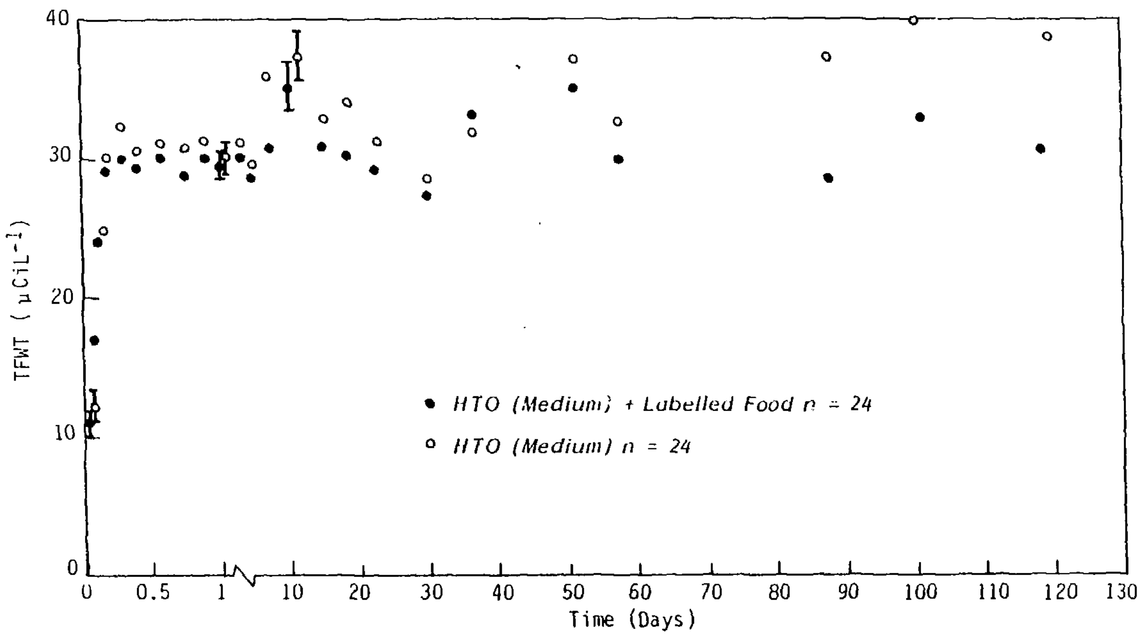

FIGURE 2

UPTAKE OF TRITIUM INTO TFWT IN ORCONECTES IMMUNIS FOR LABELLED MEDIUM AND LABELLED FOOD PLUS MEDIUM TEST CONDITIONS

In the tritiated water tests, the initial rate constant k was 19.2±5.8 day^{-1} ($\bar{x}$ ±95% C.I.), and the initial uptake rate*, $C_\infty k$, was 647 μCi L^{-1} day^{-1} (Table 1). Crayfish reared in tritiated water and food environment had a similar uptake rate constant (22.1±5.7 day^{-1}) and uptake rate 665 μCi L^{-1} day^{-1}). The steady-state concentration, C_{eq}, was not statistically different between test conditions (t_{56} = 0.8, >p 0.05) averaging 33.7±1.34 μCi L^{-1} in the tritiated water and 30.1±2.1 μCi L^{-1} in the tritiated water and food test conditions. Tritium concentration in the medium was similar for the tritiated water and food test tank (34.0 mCi L^{-1}) and the tritiated water alone test tank (38.3 mCi L^{-1}).

TABLE 1

HTO Uptake Kinetics in *Orconectes immunis* Reared in Labelled Medium, and Labelled Medium and Food Test Conditions

Experimental Condition	Rate Constant k (day^{-1})	95% C.I. Upper/ Lower	Steady-State Conc'n, C_∞ (μCi L^{-1})	95% C.I. Upper/ Lower
Tritiated Water & Food	22.1	16.4 27.7	30.1	32.2 30.0
Tritiated Water	19.2	13.4 25.0	33.7	35.0 32.3

After 4 h exposure, the mean ratio of TFWT to HTO was 0.91 ($\bar{x}$ = 0.90 tritiated water; x = 0.92 tritiated water and food), which is consistent with the steady-state reported for other aquatic invertebrates (Harrison and Koranda, 1971; Tucker and Harrison, 1974; Adams et al, 1979). Theoretically, the concentration of tissue-free water tritium (under steady-state) would be expected to equal the concentration of environmental water (ie unity).

Tissue-free water tritium estimates were also made on the exoskeleton and muscle and visceral tissues of crayfish at the termination of exposure (Table 2). In animals (live or dead) exposed to tritiated water or tritiated water and food, TFWT levels in the exoskeleton were significantly lower than those recorded in the medium (a ratio of TFWT/HTO less than 0.1). The reason for this difference is not known but may involve discrimination against tritium in the exoskeleton. The TFWT levels in muscle and visceral tissues were considerably higher than that of exoskeleton, and the ratio of TFWT to HTO approach unity.

* Initial uptake rate is a product of the rate constant, k, and the steady-state concentration, C_∞ (see Table 1).

<u>TABLE 2</u>

TISSUE-FREE WATER TRITIUM (TFWT) LEVELS RECORDED IN EXOSKELETON, MUSCLE AND VISCERAL TISSUES

Date	Experimental Condition (Tritiated)	Ambient Medium Concentration (x ±S.D.(x) μCi L^{-1})		TFWT (μCi L^{-1})		TFWT/HTO Ratio	
				Exo-skeleton	Muscle and Visceral Tissues	Exo-skeleton	Muscle and Visceral Tissues
Nov 5 (117 days in culture)	Water	38.3	(2.10)	0.81	38.6	0.02	1.01
	Water + Food	34.0	(1.82)	0.11	31.4	<0.01	0.92
July-August (dead animals) (<40 exposed days)	Water	38.3	(2.10)	0.26	37.6	0.01	0.98
	Water + Food	34.0	(1.82)	0.17	35.3	<0.01	1.04

It is noteworthy that these estimates ($\bar{x}$ = 0.99) were slightly higher than that estimated for whole animals (a ratio of 0.91).

2. Uptake Kinetics of Tritium as Organically-Bound Tritium

Uptake of tritium into the organically-bound (OBT) fraction (Figure 3) proceeded considerably slower than that observed for tissue-free water (TFWT, Figure 2). After 117 days of rearing in a tritiated environment, there was no evidence of steady-state observed in OBT levels. Highest OBT levels occurred in crayfish exposed to tritiated water and food, followed by tritiated water, and tritiated food test conditions. Organically-bound tritium levels in animals exposed to tritiated water increased near linear over the experimental period (OBT in μCi g^{-1} H = 0.0007 time (days) + 0.02, r = 0.95, 23 d.f.). In the tritiated food and water test conditions, a noticeable increase in OBT occurred 17 days after the food source (tritiated lettuce) was introduced. This increase was attributable (almost to the day) to a reduction in water temperatures in the experimental tanks (ie below 23°C, Appendix A) to levels which were more optimal to feeding conditions (Winberg, 1971), as well as a probable reduction in animal stress since animals were no longer transferred between tanks.

Over the experimental testing period, mean OBT concentrations in animals exposed to tritiated food and water were approximately 31% and 73% higher compared to animals held in tritiated water, and tritiated food test conditions, respectively. Similarly, Komatsu et al (1981) recorded OBT levels in brine shrimp significantly higher (~40%) in a tritiated food and water environment, than a tritiated water environment. In our tests, the higher contribution of OBT in crayfish from the water rather than from the food source may, in part, be attributed to a higher specific activity (p Ci g^{-1} H) of the water.

Organically-bound tritium concentrations (μCi g^{-1} H) were consistently higher in exoskeleton* than in muscle and visceral tissues (Table 3). This may be attributed to a faster growth in the exoskeleton during the time course than muscle and viscera. It is also possible that discrimination for tritium may be occurring.

* It is noteworthy that the total hydrogen content of exoskeleton (29.7 g kg^{-1}) is considerably less than muscle and tissue viscera (54.7 g kg^{-1}).

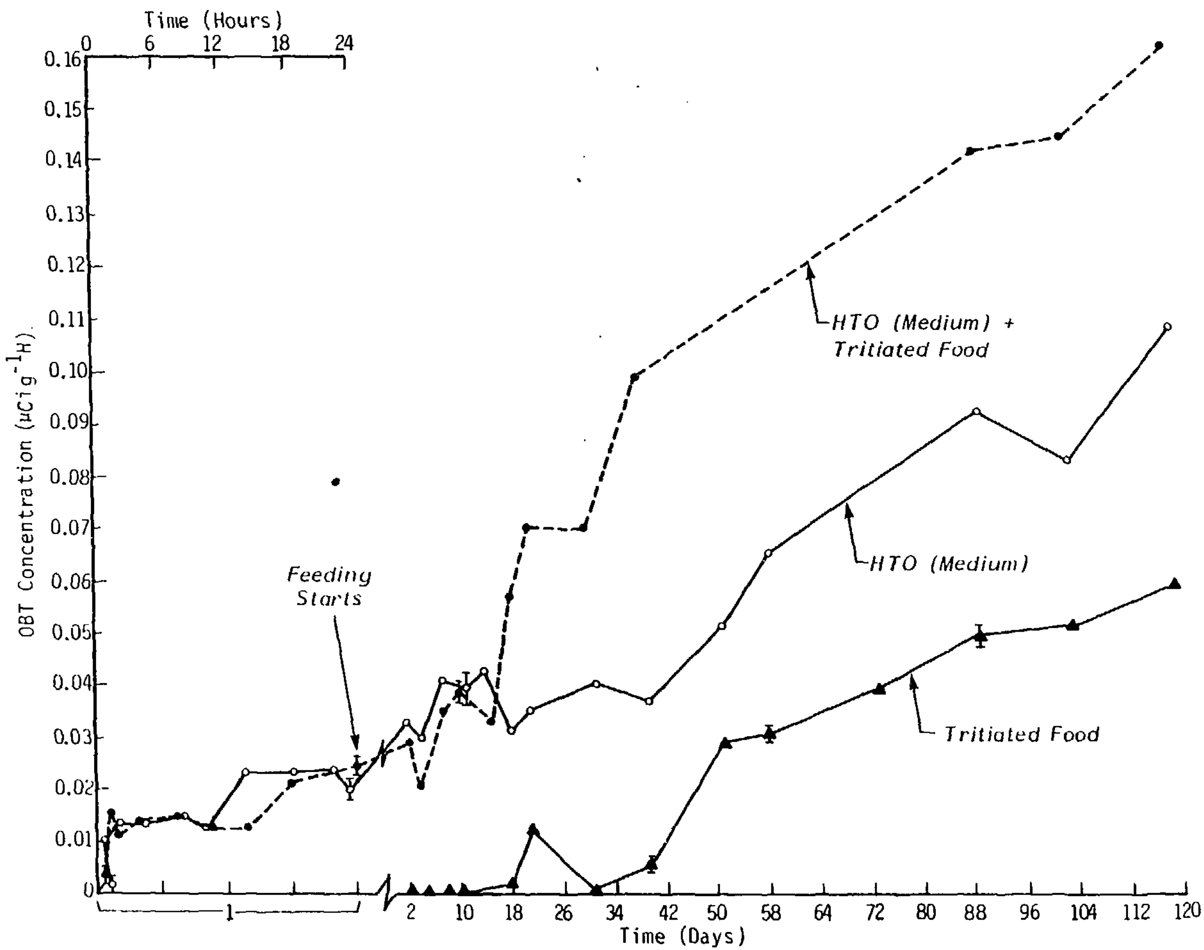

FIGURE 3

UPTAKE OF TRITIUM AS ORGANICALLY BOUND IN ORCONECTES IMMUNIS FOR VARIOUS TEST CONDITIONS

TABLE 3

ORGANICALLY-BOUND TRITIUM (OBT) LEVELS RECORDED IN EXOSKELETON, MUSCLE AND VISCERAL TISSUES

Date	Experimental Condition (Tritiated)	OBT Concentrations ($\mu Ci\ g^{-1} H$)	
		Exoskeleton	Muscle and Visceral Tissue
Nov 5 (117 days in culture)	Water	0.552	0.113
	Water + Food	0.555	0.183
July - August (dead animals) (45 day exposure)	Water	0.129	0.066
	Water + Food	0.135	0.095

Apart from water and food sources, the old exoskeleton can also serve as a source of tritium to the new exoskeleton during the moulting process, especially when feeding ceases. This may result in an accumulation of tritium in the exoskeleton via the remobilization of 'old' exoskeleton, and subsequent incorporation into 'new' exoskeleton existing at harvest time. The actual mechanisms involved are not known.

Based on the relative weight (ash free dry wt) contributions of exoskeleton (13.8%) and muscle and visceral tissue (86.2%) to the total organism, the expected OBT level for whole crayfish was estimated and compared to observed values. On average, the expected levels (based on exoskeleton, muscle and visceral tissues) were 30% higher than observed determinations on whole animals. These differences may result from variability in sampling, and also possible methodological errors in either over-estimating OBT levels in the exoskeleton or under-estimating levels in the muscle and visceral tissues.

There is little literature available to compare these OBT results. Although Harrison and Koranda (1971) noted that OBT concentrations in the exoskeleton were less than that of muscle, the crayfish used in their tests were adult, and did not moult (Harrison, pers. comm.). Their data do, however, indicate that tritium can incorporate into carapace material even though little or no growth has occurred. This may be due to some turnover and/or tritium-hydrogen exchange reactions.

3. Relationship Between TFWT and OBT

Differences were evident in the specific activity (OBT/TFWT) ratios in crayfish grown in tritiated water, and tritiated food and water test conditions (Figure 4). These differences were especially noticeable after 17 days of culture, where there was a substantial increase in OBT concentrations in the labelled medium and food test condition shown previously (Figure 3). Although steady-state was never reached, maximum OBT/TFWT ratios estimated for the tritiated food and water test condition (∿0.6) was approximately twice as high as that calculated for the tritiated water alone test condition (∿0.3). These estimates are within the range reported for other invertebrate species (Harrison and Koranda, 1971; Adams et al, 1979; Komatsu et al, 1981) where steady-state conditions were usually met. Although OBT/TFWT ratios did not exceed unity in our tests, steady-state conditions were not reached. Over a longer time period, this ratio will likely increase further, and may approach unity. In our tests, crayfish biomass probably at least doubled. Still, an order of magnitude change in biomass (ie, significant growth) may be required for OBT levels to reach steady-state. Many aquatic invertebrates experience a change in biomass of over 100-fold during thir life cycle (Winberg, 1971). Organically-bound tritium levels may also be under-estimated in our tests as a result of exchangeable tritium loss. However, this is perhaps unlikely if HTO behaves as H_2O. The magnitude of this potential is not known, but probably would not exceed 25% (estimated size of the exchangeable pool of organically-bound tritium for several invertebrate species, Hatch and Mazrimas, 1972; Tucker and Harrison, 1974).

Specific activity relationships of OBT/TFWT varied between exoskeleton, muscle and visceral tissues (Table 4). As noted above, (Tables 2 and 3), crayfish exposed to tritiated water, and tritiated food and water showed higher OBT, and lower TFWT concentrations in the exoskeleton compared to muscle and visceral tissues. As a result, the OBT/TFWT ratio in exoskeleton material far exceeded unity (Table 4), especially for animals collected on the last sampling date (117 days of culture). The OBT/TFWT ratio estimated for muscle and visceral tissue was generally equal to less than 0.3.

Specific activity relationships were also compared between the lettuce food source and crayfish. The ratio of OBT in crayfish to lettuce for whole animals, was less than or at unity for crayfish held in both tritiated water (0.6) and tritiated food and water (1.0) environments, respectively. This latter ratio is probably not significant given the variability in the data.

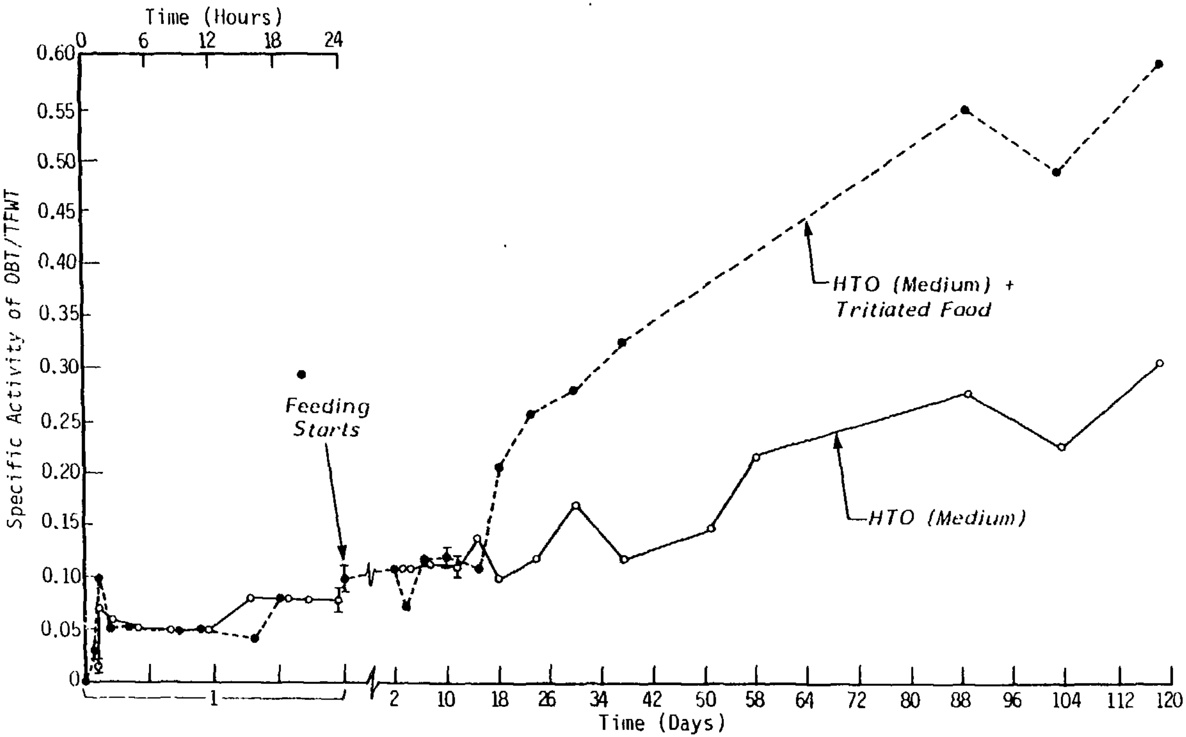

FIGURE 4

SPECIFIC ACTIVITY RELATIONSHIPS OF ORGANICALLY BOUND TRITIUM TO TISSUE-FREE WATER TRITIUM IN ORCONECTES IMMUNIS

TABLE 4

SPECIFIC ACTIVITY RELATIONSHIPS (OBT/TFWT) OF EXOSKELETON, MUSCLE AND VISCERAL TISSUES

Date	Experimental Condition	Exoskeleton (OBT/TFWT)	Muscle and Visceral Tissue (OBT/TFWT)
Nov 5 (117 days in culture)	Water	76	0.3
	Water + Food	623*	0.6
July-August (dead animals)	Water	58	0.2
	Water + Food	94	0.3

* probably an over-estimate

SUMMARY

1. The kinetics of tritiated water (HTO) uptake in *Orconectes immunis* was investigated under laboratory conditions.

2. Uptake of tritium into the tissue-free water fraction (TFWT) followed a standard exponential kinetic uptake curve. In the tritiated medium, and tritiated food and medium test conditions, uptake rate constants were 22.1±5.7 (95% C.I.) and 19.2±5.8 day^{-1}, respectively. After 4 h exposure, the ratio of TFWT/HTO was 0.90 in labelled medium, and 0.92 in labelled food and medium test conditions.

3. Uptake of tritium as organically-bound (OBT) proceeded slowly, and did not reach equilibrium up to 117 days of exposure. Highest OBT concentrations were observed in the tritiated food and water tests, which was approximately 31% and 73% higher compared to animals held in tritiated water and tritiated food test conditions, respectively.

4. Highest OBT/TFWT ratio in whole animals was approximately 0.6 but exceeded unity in the exoskeleton. The exoskeleton showed significantly lower TFWT, but higher OBT concentrations than muscle and visceral tissues.

5. Specific activity ratios of OBT between crayfish and lettuce (food source) was 0.6 and 1.0 for animals held in tritiated water only, and tritiated water and food environments respectively.

6. These results provide further support for the common assumption held by the tritium specific model with the exception of OBT/TFWT exoskeleton results which may be a function of metabolic isotopic discrimination of tritium versus hydrogen.

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APPENDIX A

TANK TEMPERATURES

TEMPERATURE (°C)

Date	Tank A (Tritiated Water + Food)	Tank B (Tritiated Water)	Tank C (Tritiated Food)
July 16	24.8	24.1	24.1
20	25.8	25.5	25.7
21	24.5	24.1	24.5
23	23.8	24.6	23.6
26	23.1	22.2	22.8
27	20.9	22.9	20.8
28	21.7	21.2	21.1
29	21.8	20.3	20.4
Aug 1	21.9	22.0	22.0
3	22.9	22.0	21.8
4	21.6	21.8	21.6
5	21.7	22.3	22.0
6	21.7	21.9	22.0
9	21.8	21.8	22.0
10	21.8	21.9	21.9
11	21.6	21.6	21.8
12	21.5	21.5	21.7
13	21.3	21.6	21.7
16	21.5	21.9	21.7
17	21.5	22.0	21.5
18	21.3	21.8	21.6
19	21.3	21.9	21.6
20	21.4	22.0	21.6
23	21.4	21.4	21.1
24	19.8	19.0	18.5
25	20.2	19.8	18.6
26	21.1	20.8	20.0
27	20.8	21.0	20.4

APPENDIX A (CONT'D)

TANK TEMPERATURES

TEMPERATURE (°C)

Date	Tank A (Tritiated Water + Food)	Tank B (Tritiated Water)	Tank C (Tritiated Food)
Aug 31	22.7	21.6	21.3
Sept 1	23.2	21.3	21.6
3	23.0	21.3	21.5
4	23.0	21.3	21.0
7	22.8	21.3	21.0
8	20.1	20.6	20.1
9	20.2	21.0	19.7
10	20.7	20.9	19.8
13	21.5	21.6	20.3
14	21.5	21.7	20.3
15	22.1	20.3	20.0
16	22.0	20.3	20.6
17	20.3	20.1	20.5
20	21.3	19.9	20.1
21	21.5	20.2	20.0
22	21.0	20.5	20.7
23	21.3	20.7	21.0
24	22.5	20.8	20.9
27	21.6	20.9	21.1
28	21.6	20.9	21.1
29	19.5	20.6	21.5
30	19.3	21.1	21.5
Oct 1-7	19.7 - 20.0	19.4 - 20.2	19.1-20.6
10-15	19.5 - 20.0	19.5 - 20.2	19.4-20.6
18-25	18.7 - 21.0	19.1 - 19.8	18.8-19.8
26-29	18.6 - 19.3	19.0 - 19.2	19.0-19.8
Nov 1-7	19.7 - 20.9	19.7 - 20.2	19.0-19.8 -20.3

APPENDIX B

Size frequency distribution of *Orconectes immunis* over the sampling period (Sampling periods shown when sample size is equal or greater than 50). The size frequency plot on November 5 may be seriously biased since it includes all remaining animals in the tank. There was a preference for selecting the larger individuals for analysis throughout the monitoring period.

FIGURE B

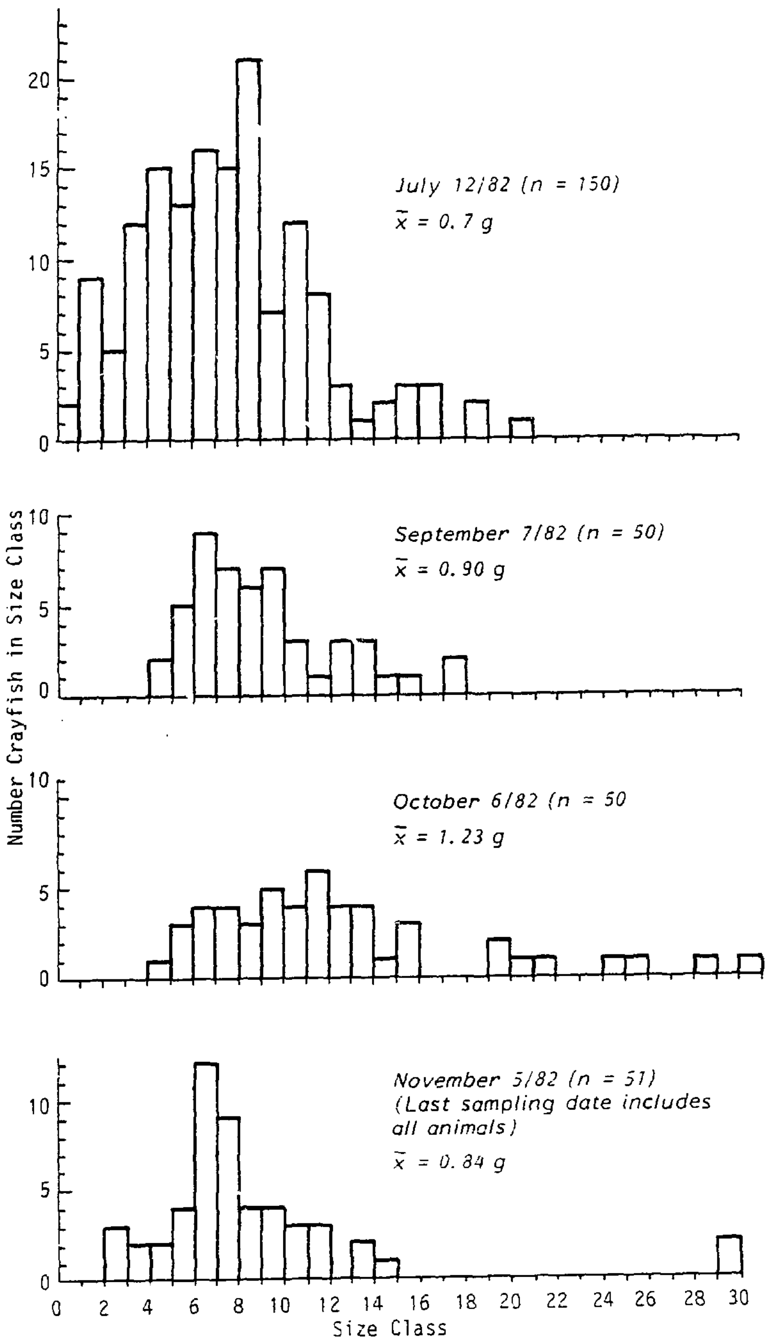

FIGURE B

SIZE FREQUENCY DISTRIBUTION OF ORCONECTES IMMUNIS OVER THE EXPERIMENTAL PERIOD

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APPENDIX C

TFWT AND OBT LEVELS IN CRAYFISH

TABLE C2 - TRITIATED WATER ENVIRONMENT

DATE	TIME (Day)	TFWT ($\mu Ci\ L^{-1}$)	OBT ($\mu Ci\ g\ H^{-1}$)
July 12-13	0.5 h	12.25	.0056
	0.5	12.66	.0029
	0.5	11.99	.0
	1.0	16.23	.010
	2.0	25.47	.013
	4.0	30.61	.014
1 day	8.0	32.82	.015
	10.0	31.81	.013
	18.0	31.91	.023
	20.0	31.68	.023
	24.0	30.49	.022
	24.0	29.93	.019
	24.0	30.67	.024
July 14	2	31.80	.033
16	4	29.67	.030
19	7	36.44	.041
22	10	33.90	.031
	10	39.68	.053
	10	40.06	.037
37	15	33.96	.042
30	18	34.82	.031
Aug 4	23	31.31	.035
10	30	28.50	.044
17	32	32.72	.036
31	51	37.92	.051
Sept 7	58	32.85	.065
Oct 7	88	36.77	.092
20	101	40.09	.083
Nov 5	117	38.71	.108

APPENDIX C

TFWT AND OBT LEVELS IN CRAYFISH

TABLE C1 TRITIATED WATER AND FOOD ENVIRONMENT

DATE	TIME (Day)	TFWT ($\mu Ci\ L^{-1}$)	OBT ($\mu Ci\ g\ H^{-1}$)
July 12-13	0.5 h	11.74	.0040
	0.5	11.31	.0035
	0.5	12.88	.0029
	1.0	17.31	.016
	2.0	24.77	.011
	4.0	29.41	.014
	8.0	30.07	.015
1 day	10.0	29.35	.012
	14.0	30.32	.012
	18.0	29.90	.021
	20.0	29.87	.079
	24.0	29.14	.024
	24.0	27.43	.025
	24.0	27.80	.024
July 14	2	30.34	.029
16	4	29.73	.020
19	7	31.81	.035
22	10	34.33	.037
	10	38.97	.046
	10	35.22	.035
37	15	31.97	.033
30	18	30.63	.057
Aug 4	23	29.51	.070
10	30	27.81	.080
17	37	33.26	.099
31	51	35.76	-
Sept 7	58	30.85	-
Oct 7	88	28.67	.141
20	101	33.31	.145
Nov 5	117	31.49	.165

APPENDIX C

TFWT AND OBT LEVELS IN CRAYFISH

TABLE C3 - TRITIATED FOOD (LETTUCE) ENVIRONMENT

DATE	TIME (Day)	TFWT ($\mu Ci\ L^{-1}$)	OBT ($\mu Ci\ g\ H^{-1}$)
July 13	1	.001	.00003
14	2	.012	0
16	4	.020	0
19	7	.023	0
22	10	.132	0
	10	.069	0
	10	.073	0
27	15	.050	0
30	18	.029	.00011
Aug 4	23	.129	.00062
10	30	.047	0
17	37	.084	.00025
	37	.064	.00024
	37	.072	.00025
31	51	.094	.00142
Sept 7	58	.005	.00145
	58	.002	.00105
	58	.003	.00162
21	72	.212	.00185
Oct 7	88	.208	.00239
	88	.321	.00218
	88	.190	-
20	101	.196	.00246
Nov 7	117	.384	.00279