

EMRAS Tritium/C14 Working Group

THE MUSSEL UPTAKE SCENARIO

Inter-model Comparison of Tritium Concentrations in Freshwater Barnes Mussels (*Elliptio complanata*) Following an Abrupt Increase in Ambient Tritium Exposure Conditions

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1. BACKGROUND AND OBJECTIVES

Although steady-state models often provide practical tools to estimate free-water tritium (HTO) concentrations (and to a lesser extent, organically bound tritium (OBT) concentrations) (e.g. Kim et al., 2004; Kotzer et al., 2001; Kotzer and Yankovich, 2001; Adams et al., 1979; Blaylock and Frank, 1979), aquatic organisms are occasionally exposed to short-term, elevated tritium concentrations in watersheds that have fluctuating tritium levels. Depending upon the nature and the duration of such events, in some cases, steady-state models may or may not be predictive of actual organism tritium concentrations (e.g. Kotzer et al., 2001). Therefore, it is important to calibrate organisms that might serve as tritium biomonitors, in order to interpret organism responses to such fluctuations in terms of their exposure levels to tritium, as well as their biological responses (Campbell et al., 1988; Elder and Collins, 1991; Phillips, 1977; Phillips, 1980).

In general, the rates of HTO uptake and OBT formation are not well known under dynamic exposure conditions, but can be studied by transplanting biomonitoring species, such as freshwater mussels, from areas with low tritium concentrations to areas with elevated tritium levels (Bayne *et al.*, 1985; Clarke, 1981; Curry, 1977/78; Dechno and Luoma, 1992; Elder and Collins, 1991; Jackim *et al.*, 1977; Greig *et al.*, 1975; Hinch and Stephenson, 1987; Kauss *et al.*, 1981; Kauss and Hamdy, 1985; Lobel *et al.*, 1982; Lobel and Wright, 1982; Luten *et al.*, 1986; Matteson, 1948; Phillips, 1979; Rubenstein *et al.*, 1983; Servos *et al.*, 1987; Tatum, 1986; Tessier *et al.*, 1984; Tessier *et al.*, 1993). In this way, changes in HTO and OBT concentrations can be monitored to quantify responses to dynamic exposure conditions.

This was accomplished in the present study through an experiment involving the transplantation of mussels in cages from areas with low tritium concentrations to Perch Lake, a small Canadian Shield lake located on AECL's Chalk River Laboratories site with an aquatic tritium concentration of approximately 4,500 Bq/L (Kim et al., 2004; Yankovich and Kim, 2005; Yankovich et al., 2006). The results of this study provided the observations for a model validation scenario for the EMRAS Tritium/C14 Working Group.

1.1 Scenario Objective

The objective of this scenario was to conduct a model validation exercise to compare observed temporal changes in HTO and OBT concentrations in freshwater Barnes mussel (*Elliptio complanata*) tissues to predicted concentrations in response to abrupt increases in tritium exposure levels. Modelled values were calculated for scenarios in which mussels were exposed to tritium through the water pathway alone, or through both water and sediments.

2. SCENARIO DESCRIPTION

A detailed scenario description, which was developed and provided to the EMRAS Tritium/C14 Working Group (Yankovich and Kim, 2005), can be found in Appendix A. The scenario focused on the prediction of dynamic tritium data that were measured in freshwater Barnes mussel (*Elliptio complanata*) tissues at discrete time points following transplantation from a site with background tritium concentrations to Perch Lake.

2.1 Site Description

Perch Lake (Figure 1) is situated downstream of two historic CRL Waste Management Areas (WMAs) on Atomic Energy of Canada Limited (AECL)'s Chalk River Laboratories (CRL) site. The lake receives inputs of tritium via groundwater that migrates to surface streams and the lake from these WMAs. Surface water enters Perch Lake via five small inflowing streams through gauged weirs at Inlets 1, 2, 3, 4 and 5. Surface water leaves the lake through one outflowing stream (Perch Creek) at Perch Lake Outlet (Merritt and Risto, 1975; Slater, 1975).

Tritium, in the form of HTO, enters the lake primarily via groundwater discharge through the underlying lake sediments and through surface water at the Inlet 2 inflowing stream. The stream at Inlet 1 also has slightly elevated levels of tritium, whereas inflowing streams at Inlets 3, 4 and 5 have relatively low levels of tritium (Niemi, 2005). The spatial distribution of HTO in the lake is not known quantitatively, although it is believed that the lake is well-mixed, with a mean tritium concentration of approximately 4,500 Bq/L in the vicinity of the mussel transplantation cages. The cages were deployed in a shallow, sandy substrate where many mussels can be found naturally.

2.2 Model Input Data

Input data summarizing initial tritium concentrations in mussel tissues and environmental media, temporal changes in Perch Lake water temperatures, and mussel sizes both at the time of transplantation and at the time of harvest were provided to modellers participating in the scenario. These input data have been compiled in Table 1 to Table 5.

A summary of the experimental methodologies that were applied to generate the measured values, and the uncertainty surrounding them, is provided in Section 3 below.

3. OBSERVATIONS

3.1 Study Design

Two pairs of mussel transplantation cages were built and deployed in Perch Lake in early 2004 July. The transplantation cages had dimensions of 96 cm (length) x 96 cm (width) x 12 cm (height) and were constructed using 2" x 2" cedar boards and chicken wire. Each cage was built with an 8 x 8 design, resulting in a total of 64 compartments per cage (Yankovich and Kim, 2005). Individual cage compartments had surface area dimensions of 12 cm x 12 cm with one animal per compartment to provide the mussels with adequate space without overcrowding.

Test mussels were collected on 2004 July 5 to 7 from a nearby reference site in the Ottawa River (Figure 1) where tritium concentrations were less than 10 Bq/L HTO in water. During collection, each mussel was carefully examined to assess its suitability for the study. Mussels with total shell lengths of 90 to 111 mm were selected to standardize size and filtration rates, to ensure adequate tissue biomass for tritium analysis and to take account of the dominant mussel size distribution that was present at the background site to reduce the sampling time required. Damaged or unhealthy mussels (e.g. those incapable of closing their shells) were not selected. HTO concentrations in the soft tissues of the reference mussels were less than 10 Bq/L. OBT levels were less than 15 Bq/L for mussels that were frozen immediately after collection, and 45 Bq/L for mussels that were stored in lidded buckets at the CRL site over a period of three days.

Upon collection, mussels were placed into lidded, plastic buckets containing water from the reference site to prevent uptake of tritium prior to initiation of the study. The mussels were then transported to the CRL site and individuals were quickly measured, weighed and alpha-numerically numbered with a cage number and cage compartment number using a DremelTM engraver for tracking purposes. Labelled clams were separated by placing them into labelled nylon bags and replaced into the lidded buckets of water from the reference site until initiation of the transplantation into Perch Lake, which was carried out on the same day as mussel collection.

Two sets of exposure conditions were established in Perch Lake. These included exposure to tritium via the surface water pathway only (Cages 1 and 2), and exposure via both sediments and surface water (Cages 3 and 4). Cages 1 and 2 were positioned on cement blocks at a depth of approximately 0.75 m, whereas Cages 3 and 4 were placed at the sediment-to-water interface at a depth of approximately 0.4 m. Each compartment in Cages 3 and 4 was filled with 5 to 10 cm of sandy surface sediments originating from the area surrounding the cages, a depth that enabled mussels to position themselves in an upright position with their siphons pointed upwards, as they do in natural systems. The sediments were added to the cages several hours prior to transplantation of the mussels to allow settling of any suspended particulates.

Mussel Cages 1 and 2 were deployed in Perch Lake on 2004 July 5 at 14:00 hours, whereas Cages 3 and 4 were deployed on 2004 July 7 at 14:00 hours. Upon initiation of mussel transplantation (at time 0), individuals were first transferred from the buckets containing water from the reference site to buckets containing water from Perch Lake, such that all mussels received initial tritium exposure at approximately the same time, despite the 10 to 15 minute time period required to transfer all the mussels from buckets to the numbered cage compartments.

Following transplantation into the cage compartments, mussels were visually monitored. In general, in Cages 3 and 4 (which contained sediments), mussels began positioning themselves in an upright position within five minutes of transplantation. In addition, after being placed into the cage compartments, the mussels in Cages 1 and 2 (without sediments) began filtering within less than five minutes. No mussel mortality occurred in any of the four cages over the course of the 86- to 88-day study.

Whole-mussel fresh weights were measured just prior to transplantation, as well as following mussel harvest (Table 2, Yankovich and Kim, 2005). In general, mussels did not show increased weight gain over the course of the study, as indicated by an arithmetic mean post-harvest-to-initial mussel fresh weight ratio of 0.981. This lack of mussel growth was not surprising, since the mussels used in this study were likely 14 to 15 years old (e.g. Negus, 1966). The small weight losses that were noted for some individual mussels may have been due to the fact that the mussels were processed while they were still frozen (to prevent exchange of mussel free-water tritium with the atmosphere) and some water loss may have occurred as ice was lost from mussel tissues. In addition, it is possible that some weight loss occurred as female mussels released their eggs during reproduction.

3.2 Generation of Model Input Data – Experimental Methodologies and Observations

3.2.1 Monitoring of Water Temperatures in Perch Lake

Perch Lake surface water temperatures were recorded continuously during 2004 July to October using a Model 107b Campbell Scientific Inc. Temperature Probe and data logger set to integrate values over 5 minute time intervals (Figure 2) (Yankovich and Kim, 2005). The probe was positioned in the vicinity of the mussel cages, a few centimetres above the sediment-water interface.

Surface water temperatures were provided to modellers as input to their models (Yankovich and Kim, 2005). In general, mean monthly surface water temperatures (\pm standard error) of 22.3 ± 0.25 °C, 16.7 ± 0.16 °C, 14.9 ± 0.10 °C and 13.8 ± 0.03 °C were measured in Perch Lake in 2004 July, August, September and October, respectively. Corresponding air temperatures measured at the ground surface showed a fairly similar trend, with mean monthly values (\pm standard error) of 20.1 ± 0.27 °C, 17.8 ± 0.33 °C, 16.1 ± 0.40 °C and 10.0 ± 0.38 °C, representing water-to-air temperature ratios of 1.11, 0.94, 0.93 and 1.38 for July, August, September and October, respectively (Table 1). Water temperature measurements were not available over the period from

2004 September 11 to 17, although in general, water temperature corresponded well with air temperatures (Figure 2), which were available when data gaps occurred for the water.

3.2.2 *Sample Collection and Processing*

Surface water, sediment and mussel samples were collected on an expanding time-step over the course of the study period (Table 3 to Table 5). Upon collection, mussels were immediately placed into air-tight mason jars to avoid tritium exchange with the atmosphere, and the jars containing the mussels were frozen until processing for tritium analysis could be carried out. In general, it was necessary to composite soft tissues from 3 to 4 individual mussels to reach the biomass required for HTO and OBT analysis. The mean water content of mussel tissue was 89% (by weight), with little variability among individual animals.

Both water and sediment samples were collected in triplicate at each sampling time in the vicinity of each of the mussel cages. In doing so, water sample bottles were opened at the depth where the mussels were filtering and the samples were subsequently left standing for at least 4 hours to allow suspended sediments to settle.

Sediment samples were collected by hand at a depth of 5 to 10 cm and were placed in Ziplock™ bags that were sealed at depth. Sediment porewater was extracted from a subset of these samples by freeze-drying at a pressure between 10^{-4} and 10^{-5} Torr and a temperature of 0 to -4° C, and the porewater was analyzed for HTO concentration by liquid scintillation counting (LSC). The remaining solid sediment material was washed with tritium-free water to remove the exchangeable OBT. Sediments were oven-dried until no change in mass occurred and dried sediments were combusted in a combustion tube with oxygen flow. The combustion water was analyzed by LSC to determine OBT concentrations, which served as input data for the scenario.

Plankton samples were collected in the Perch Lake water column on 2004 September 20 just offshore of the mussel cages to quantify tritium levels in mussel dietary items (as an input parameter for modeling purposes). HTO levels of 4153, 4101 and 4068 Bq/L were measured in the plankton samples. Corresponding HTO concentrations in Perch Lake surface waters at the time of plankton sampling were 4091, 4066 and 4038 Bq/L, respectively.

It was not possible to measure OBT in individual plankton samples due to the relatively large biomass required for OBT analysis and the relatively large water content present in plankton samples. As a result, OBT levels were measured in a single composite plankton sample, which had a value of $2,914 \pm 42$ Bq/L.

3.2.3 *Sample Tritium Analyses*

3.2.3.1 HTO Analysis

HTO concentrations were analyzed in water, sediment, mussel and plankton samples collected in 2004 (Table 3 to Table 5).

Surface water samples were analyzed for tritium in accordance with a standard procedure that has been developed by AECL (ETB-ERM-602 Rev 2.2, 1999). Briefly, 2 mL of water sample were mixed with 10 mL of Ultima Gold scintillation cocktail and placed in a 20 mL polyethylene PackardTM scintillation vial (Workman and Brown 1992; Workman 1999; Workman 2000). The samples were then counted for 30 minutes using a Beckman 6500 LSC in AECL's environmental laboratories in Building 513 (B513) at CRL. Tritium analysis of a few background samples was performed at AECL's Low Background Environmental Laboratory (Building 560, (B560)) using a Quantulus 1220 LSC (Wallac, Finland). The lower limits of detection (LLD) for the HTO measurements were approximately 1.0 Bq/L for the Quantulus and 60 Bq/L for the Beckman LSC.

The free-water (HTO) of the mussels, plankton and sediment samples was extracted using a freeze-drying system in AECL's environmental labs in B513 at CRL or in B560, depending upon the expected HTO level in a given sample. For example, mussels collected within 24 hours of transplantation were analyzed in B560, whereas other samples, including Perch Lake sediments, were analyzed in B513. The samples were loaded into vacuum flasks and exposed to dry ice traps at vacuum pressure for 24 hours. Incompletely dried samples were placed in a drying oven at 60°C for 24 hours. Tritium concentrations in the free-water were determined using the Quantulus 1220 LSC in B560 or the Beckman 6500 in B513. For the Quantulus LSC, 10 mL of water were mixed with 10 mL of Ultima Gold cocktail.

3.2.3.2 OBT Analysis

Mussel and sediment dry matter remaining after the HTO analysis was chopped and homogenized using scissors and mixed with 30 to 50 mL of tritium-free-water to remove the exchangeable OBT. The samples were then refrozen and subjected to a second round of cryogenic distillation under vacuum. This process was repeated at least twice, until the tritium concentration of the rinse-water was less than 4.0 Bq/L. Most samples reached this value following the second rinse. The completely rinsed mussel samples were then combusted using a Parr bomb with pressurized oxygen. The sediment samples were combusted using a furnace type combustion tube with oxygen flow. Samples collected within 24 hours of transplantation were measured for OBT at B560. The combusted water from these samples was made up to 10 mL with tritium-depleted water and combined with 10 mL of Ultima Gold XR to measure the OBT concentrations. OBT in the remaining samples (that had been collected more than 24 hours after transplantation) were measured in B513. In such cases, 2 mL of the combustion water were mixed with 10 mL of Ultima Gold AB (Perkin-Elmer).

Counting errors for OBT concentrations were generally less than 5%, but additional uncertainty arose due to difficulties in removing exchangeable OBT from the samples and in the combustion process. The total uncertainty in the OBT measurements is estimated to be approximately 25%.

4. MODEL DESCRIPTIONS

A total of five models from Japan, Romania (Galeriu et al., 2005), France and Germany (Baumgärtner, 2000 and 2005; Baumgärtner and Donhaerl, 2004; Baumgärtner and Kim, 2000) participated in the mussel uptake scenario (Table 6). The modelling teams were asked to predict temporal changes in mussel HTO and OBT concentrations, along with the 95% confidence intervals on each model prediction, using the model input data that were provided.

A summary of the key assumptions of each modelling approach is provided in Table 7; detailed descriptions of each model can be found in Appendix B.

5. RESULTS AND DISCUSSION

5.1 Modelled-to-Measured Comparisons

5.1.1 Prediction of Mussel HTO

Both the NIRS and SRA models under-predicted HTO uptake rates over the initial 8 days of the study period, after which the values predicted by NIRS fell within 1.04- to 1.23-fold of the measured values and the values predicted by SRA fell within 1.01- to 1.08-fold of the observations for all mussel cages (Figure 3).

By comparison, mussel HTO concentrations that were predicted using AQUATRIT were very close to measured values at all sampling time points, with modelled-to-measured ratios that ranged from 0.7 to 1.2 (for Cages 1 and 2) and from 0.8 to 1.2 (for Cages 3 and 4) (Figure 3).

With the exception of the one-hour time point for Cages 3 and 4 (for which predicted values were approximately 1.5-fold higher than measured values), the EDF model showed good predictive power throughout the study, with modelled-to-measured ratios that ranged from 1.03 to 1.15. Similarly, with the exception of the Cage 3 and 4 one-hour time point (which showed a modelled-to-measured ratio of 1.6), the BIOCHEM model predictions fell within 1.01- to 1.15-fold of the measured values.

5.1.2 Prediction of Mussel OBT

The NIRS model initially under-estimated mussel OBT concentrations by 2- to 6-fold, then over-estimated the measured values by 2- to 7-fold, and finally began to approach the measured values 36 after transplantation (Figure 4).

The SRA model also initially under-estimated OBT until approximately 14 days (for Cages 3 and 4) to 18 days (for Cages 1 and 2) had passed, after which predicted values fell close to measured values. Between 18 and 42 days, modelled-to-measured OBT ratios lay between 0.7 and 0.9 for all mussel cages, although SRA slightly over-estimated

mussel OBT concentrations on Day 86, showing modelled-to-measured ratios of approximately 1.7 (for Cages 1 and 2) and 2.3 (for Cages 3 and 4), respectively (Figure 4).

OBT concentrations in mussels receiving tritium exposure via water only (i.e., Cages 1 and 2) were under-estimated by the AQUATRIT model until almost the end of the study period by factors of 210 after 1 hour to 1.3 after 86 days (Figure 4). By comparison, in general, AQUATRIT OBT predictions for mussels exposed to tritium via both water and sediments (Cages 3 and 4) were closer to measured values, particularly when it was assumed that mussels were feeding on food (e.g., plankton) that was at steady state with respect to HTO levels in Perch Lake. Predictions based on this assumption are reflected by the 'corrected' OBT predictions for the AQUATRIT model, whereas 'uncorrected' OBT predictions do not account for the presence of tritium in the mussel food source (Figure 4 and Figure 5). In general, uncorrected OBT predictions for mussels receiving tritium exposure via water plus sediments had modelled-to-measured OBT concentrations that fell between 0.02 and 0.8 (excluding the 86 day time point, which was over-estimated by most models), whereas those that had been corrected for food based on Perch Lake HTO concentrations ranged from 0.3 to 1.06 (Figure 4).

OBT concentrations were under-estimated by the EDF model by approximately 2- to 3-fold during the first four hours following transplantation. Thereafter, EDF model predictions were very close to measured values and remained so until the last data point, which was over-estimated by most models.

Over the first day, the BIOCHEM model initially slightly under-estimated mussel OBT concentrations by factors of approximately 1.5- to 2-fold (Figure 4). After the first day, modelled-to-measured OBT ratios of 0.7 to 1.1 were predicted by BIOCHEM.

5.1.3 Inter-Model Comparisons

It is not only important to appraise the similarities and differences in predicted HTO and OBT concentrations at a given time point, but also the rate of increase of these concentrations over time. This was done through linear regression analysis (Table 8 and Table 9). In addition, analysis of covariance (ANCOVA) was conducted to test whether or not there were significant differences in slope (which reflects the rate of change of modelled-to-measured HTO or OBT concentration in mussel tissues with time after transplantation).

5.2 Tritium Dynamics

5.2.1 Mussel HTO Dynamics

With the exception of the BIOCHEM model predictions, no statistically significant difference in mussel HTO concentration was predicted between mussels receiving tritium exposure from water only (in Cages 1 and 2) or from water plus sediments (in Cages 3 and 4) (Table 8).

For the initial time period when the observed rate of change of HTO concentration was relatively constant, EDF and BIOCHEM model results did not differ statistically in their predicted rates of HTO increase for mussels receiving exposure via water only (ANCOVA, $p = 0.742$; Table 8; Figure 3). Rates of HTO increase in Cage 1 and 2 mussels were significantly different for all other models, with slopes of 0.97, 0.88, 0.15, 0.037 and 0.029 for the SRA, NIRS, AQUATRIT, BIOCHEM and EDF models, respectively (Table 8).

Similarly, for mussels that had been exposed to tritium via both water and sediments (in Cages 3 and 4), the initial rates of HTO increase in mussel soft tissues differed significantly between all models (ANCOVA, $p \leq 0.0224$), with slopes of 0.94, 0.87, 0.13, 0.037 and -0.010 for the SRA, NIRS, AQUATRIT, EDF and BIOCHEM models, respectively (Table 8).

In addition, it is interesting to note that for all models, the rate of HTO increase between the 1-hour and 2-hour time points was predicted to occur relatively more slowly in mussels that received tritium exposure via both water and sediments than for mussels that were exposed to tritium via water only (Figure 3).

5.2.2 Mussel OBT Dynamics

Although all models tended to predict similar final mussel HTO concentrations regardless of whether mussels received tritium exposure from water only or from water and sediments, the same was not necessarily true for all models with respect to the prediction of mussel OBT (Figure 4).

5.2.2.1 Water-Only Pathway

In most cases, the predicted patterns of mussel OBT formation for exposure to water only (Cages 1 and 2) were similar among all models (Figure 4). Despite this similarity, however, the initial rates of OBT formation and the initial OBT concentrations in mussel tissues often greatly differed among the models, as reflected by relatively large differences in the y-intercepts on plots of OBT concentration versus time (Table 9). For example, although the initial rates of OBT increase did not significantly differ for the AQUATRIT and BIOCHEM models, BIOCHEM estimated an initial mussel OBT concentration of 0.63 one hour after transplantation, compared to a y-intercept value of 0.08 for AQUATRIT. This represents an 8-fold difference between the initial OBT predictions of the two models. This suggests that the initial conditions assumed by a

particular model can play a significant role in determining the initial concentration and possibly the predicted rate of increase in the concentration.

5.2.2.2 Sediment Plus Water Pathways

Similar trends and relative OBT formation rates were found between Cages 1 and 2 (water only) and Cages 3 and 4 (water plus sediments) for most models. This suggests that the models did not predict significant differences in OBT formation, regardless of exposure pathway (Table 9). The exception was the two AQUATRIT runs that predicted OBT in mussels consuming uncontaminated food and food with tritium levels that reflected Perch Lake tritium concentrations.

As for HTO, for all models, the rate of OBT formation between the 1-hour and 2-hour time points was predicted to occur relatively more slowly in mussels that received tritium exposure via both water and sediments than via water only (Figure 4). Similar trends were also predicted by the EDF and BIOCHEM models for mussels receiving tritium exposure via water only, as indicated by the relatively less steep slope between the 1-hour and 2-hour time points compared to later times (Figure 4).

Evaluation of the rate of change of OBT concentration over time indicated that no significant differences in the initial rates of OBT formation occurred between the SRA and AQUATRIT models, although significant differences existed in the predictions of all other models.

In addition to the rate of change of OBT formation, it is also important to compare the initial starting conditions (or the y-intercepts) that are predicted by each model in the linear range when plotting changes in modelled-to-measured OBT concentrations in mussels over time. In general, although the rate (or slope) of OBT formation did not differ between the SRA and AQUATRIT models, the assumed starting conditions did (assuming that mussels were consuming contaminated food items), with y-intercepts of 0.276 and 0.987, respectively (Table 9). This suggests that, although mussel starting conditions were not assumed to be the same, the factors leading to OBT formation may have been similar. A range of y-intercepts were predicted for Cage 3 and 4 mussels by the NIRS, EDF and AQUATRIT models, with values of 5.87, 1.59 and 0.630, respectively. By comparison, similar y-intercepts were predicted by the SRA model and the AQUATRIT model run that assumed mussels were consuming uncontaminated food (Table 9).

5.3 Pathways Analysis for Tritium Uptake by Mussels

In general, with the exception of individual predictions that were made using the AQUATRIT, EDF and BIOCHEM models, mussel HTO uptake was predicted to be similar for mussels exposed to HTO via water only (in Cages 1 and 2) and those exposed via both water and sediments (in Cages 3 and 4) (Figure 5). In the exceptional cases, which tended to occur within the first hour of transplantation, a relatively higher HTO uptake was predicted for mussels that had been added to the cages where they had access

to both sediments and water. It is possible that these mussels (which were exposed to higher suspended matter content) were initially filtering more slowly as they took time to optimally position themselves in the sediments and made use of available suspended matter, whereas mussels exposed to water only began to filter more quickly upon transplantation. This may have resulted in the slight over-estimation of mussel HTO levels at the first time point predicted by the AQUATRIT, EDF and BIOCHEM models for animals exposed to water plus sediments relative to those exposed to water only.

With the exception of the two AQUATRIT model runs, predicted OBT uptake was similar when mussels were exposed to tritium via water alone compared to when they were exposed via both water and sediments (Table 9; Figure 5). However, it is interesting to note that for all models, when mussels received tritium exposure via both water and sediment, the rate of uptake was initially predicted to be slower than when they were exposed via water only (Figure 3 and Figure 4).

In the case of the AQUATRIT model (for both the scenario that assumed uncontaminated food and the scenario in which tritium concentrations in mussel dietary items were assumed to be at steady state with those in Perch Lake), it appears that some OBT contribution from the sediments was assumed. This suggests that predicted OBT concentrations for mussels receiving tritium exposure via both water and sediments were higher than those predicted for mussels receiving tritium exposure from water only (Figure 5). This concurs with the similarity in assumed initial starting conditions for tritium concentration among the models (with the exception of AQUATRIT) for Cages 1 and 2 and for Cages 3 and 4, as indicated by the similarities in the y-intercepts between the different cage conditions (Table 9). However, for AQUATRIT, when it was assumed that mussels consumed uncontaminated food, OBT levels in mussels that received tritium exposure via water only were proportional to those in mussels that receive exposure via both water and sediments (Figure 5). In comparison, such a relationship did not exist when mussels were assumed to assimilate dietary items that contained tritium at Perch Lake levels. Instead, in the latter case, it seemed that mussel OBT levels were being driven by the concentration in the sediments, and remained relatively constant with respect to changes in OBT concentrations in mussels that received exposure via water only.

6. SUMMARY AND CONCLUSIONS

In general, a number of consistencies in model predictions, in terms of either the under-estimation or the over-estimation of measured HTO and/or OBT concentrations, were identified, as discussed in the sections that follow. Such under- and over-predictions were evaluated, to the extent possible, to determine whether they could be attributed to similarities in tritium transfer or formation coefficients leading to differences between modelled and measured values, or whether they were due to unexpected fluctuations in measured data as influenced by analytical or biological factors.

6.1 Under-Estimates of Initial Tritium Accumulation Rates

In all cases where mussels received tritium exposure via both sediments and water and in a number of cases where exposure occurred via water only, the rate of HTO and OBT accumulation by mussels was relatively slow between the 1-hour and 2-hour sampling time points (Figure 3 and Figure 4).

6.2 Over-Estimates of OBT Concentrations in Mussels at the Final Time Point

With the exception of the BIOCHEM model (with assumed OBT loss during reproduction) and Cage 1 and 2 mussels for AQUATRIT (which under-estimated OBT at all time points), all models over-estimated mussel OBT concentrations at the final experimental time point (Figure 4). Evaluation of measured data indicates that these over-estimations were likely related to unexpectedly low measured OBT levels in harvested mussels at the last sampling point (Yankovich et al., 2006).

These lower-than-expected OBT concentrations may be attributed to a number of factors possibly related to mussel biology. As discussed in Section 3.1 above, mussels selected for use in the transplantation study ranged from approximately 90 to 111 mm total shell length. Based on available literature data on mussel length-to-age relationships (e.g. Negus, 1966), it is likely that the mussels collected for this study were more than 14 years old. Mussel growth typically occurs between April and September and depends upon water temperature, food availability, water currents and water chemistry. Since unionid mussels (Family Unionidae) such as *Elliptio complanata* typically reach sexual maturity between 6 and 12 years of age, it is likely that the transplanted mussels were sexually mature and, due to their relatively large size, it would be expected that the test animals were likely expending a relatively large proportion of their energy towards reproduction, as opposed to growth of somatic tissues. This could be indirectly confirmed through consideration of the mussel reproductive cycle and the mussel growth data collected over the course of the study, as well as the timing of mussel sampling with respect to the relatively sudden decline in mussel OBT at the last data point.

In terms of reproduction, unionid mussels have separate sexes. During reproduction, the unfertilized eggs are deposited into the water tubes of the gills of the females and the males release their sperm into the water column. The sperm is then drawn in by the females, allowing the eggs to become fertilized. The embryos are retained inside the females for a short period during their early stages of development, representing a period of rapid growth. Therefore, it is possible that following transplantation into Perch Lake, growing tissues, such as those of gonad tissues and mussel embryos, would incorporate tritium at a faster rate than other tissues.

Unlike other families of freshwater mussels, Unionidae are considered short-term breeders and are gravid between April and August (as opposed to long-term breeders, which fertilize their eggs in mid-summer and carry them until the following spring or summer). Mussel larvae, or glochidia, are released by females into the water column and later become a temporary, but obligatory, parasite on fish. They then leave the host fish

and deposit in the lake sediments as juvenile mussels. Therefore, it is possible that OBT was formed in reproductive tissue following mussel transplantation into Perch Lake; however, with release of glochidia into the water column between mid-August (when the second last mussel sample was taken) and early October (which represented the final sampling point), mean OBT levels in the mussels declined. In addition, the fresh weights of individual mussels just prior to transplantation relative to those at time of harvest indicate a lack of growth, and in some cases, slight declines in mussel fresh weights over the course of the study, suggesting that the female mussels lost weight with the release of larvae into the water column.

6.3 Variability in Model Predictions and Future Work

A number of factors could potentially account for the observed differences between modelled and measured HTO and OBT concentrations in the mussels. These include differences in the assumed initial starting conditions to which the transplanted mussels were exposed; the assumed HTO transfer rate into mussel tissue (which could, in turn, be influenced by physical factors such as diffusion rates and/or concentration gradients, as well as biological properties such as mussel filtration rates); assumed OBT formation rates; the expected importance of various tritium exposure pathways in terms of the HTO and OBT inventories in mussel tissues; and/or assumptions with respect to tritium speciation in the body in key biological compartments and the relative importance of these tritium species and compartments. Such factors may or may not be captured by all models, and in cases where similar processes are assumed to occur, numerical values of relevant transfer parameters may or may not be the same between models.

Accumulation processes for OBT are complex relative to those for HTO, and can be influenced by exposure pathway (which may include OBT formation following uptake of HTO and/or direct dietary uptake), as well as physiological metabolism, whereby HTO diffuses into cells and is subsequently converted to OBT. Such OBT formation mechanisms likely depend more upon initial exposure conditions than on those that obtain later on.

Furthermore, the tritium community is becoming aware of several new issues based on the findings of HTO exposure experiments in plants. Recently, new OBT species (buried tritium and hydrate-bound tritium), as well as the distinction between different OBT formation rates (i.e., fast versus slow) have been suggested as factors that should be considered in predicting tritium doses to humans and biota (Baumgärtner, 2000 and 2005; Baumgärtner and Donhaerl, 2004; Baumgärtner and Kim, 2000). For example, the BIOCHEM model is focussed on the estimation of buried tritium, as well as physical diffusion processes that can influence OBT levels in mussel tissues. In doing so, the model assumes that contributions of carbohydrate (and carbon-bound tritium) to the total OBT inventory in mussel tissues are negligible, and that OBT uptake through dietary pathways (such as ingestion of plankton) is insignificant due to the lack of mussel growth. Although only buried tritium is considered in the BIOCHEM model, the model still over-estimates total mussel OBT concentrations. This implies that buried tritium represents the dominant form of OBT in mussels. Further experimental work is clearly

required to confirm these assumptions. In addition, the uncertainty in OBT analysis is estimated to be larger than expected previously.

Such factors, as well as the way in which each model accounts for each factor, may explain the variability among model predictions, particularly for OBT. To date, only a few experiments have been designed to validate the various models and, in order to improve the understanding of tritium, and especially OBT, behaviour in abiotic and biotic environments, more scenarios and accurate datasets are required.

Future work could focus on characterization of key parameter values, such as the biological half-life of OBT, OBT formation rates over various time scales and the influence of exposure pathway on OBT accumulation, which may influence OBT concentrations in freshwater biota. Furthermore, uncertainty and sensitivity analysis, particularly for the long-term context, are required to gain additional insights into the key parameters that should be included in OBT models. However, as a necessary first step, further work is underway to gain understanding of the similarities and differences in the parameter values and the assumptions that have been applied in running the models that participated in this scenario.

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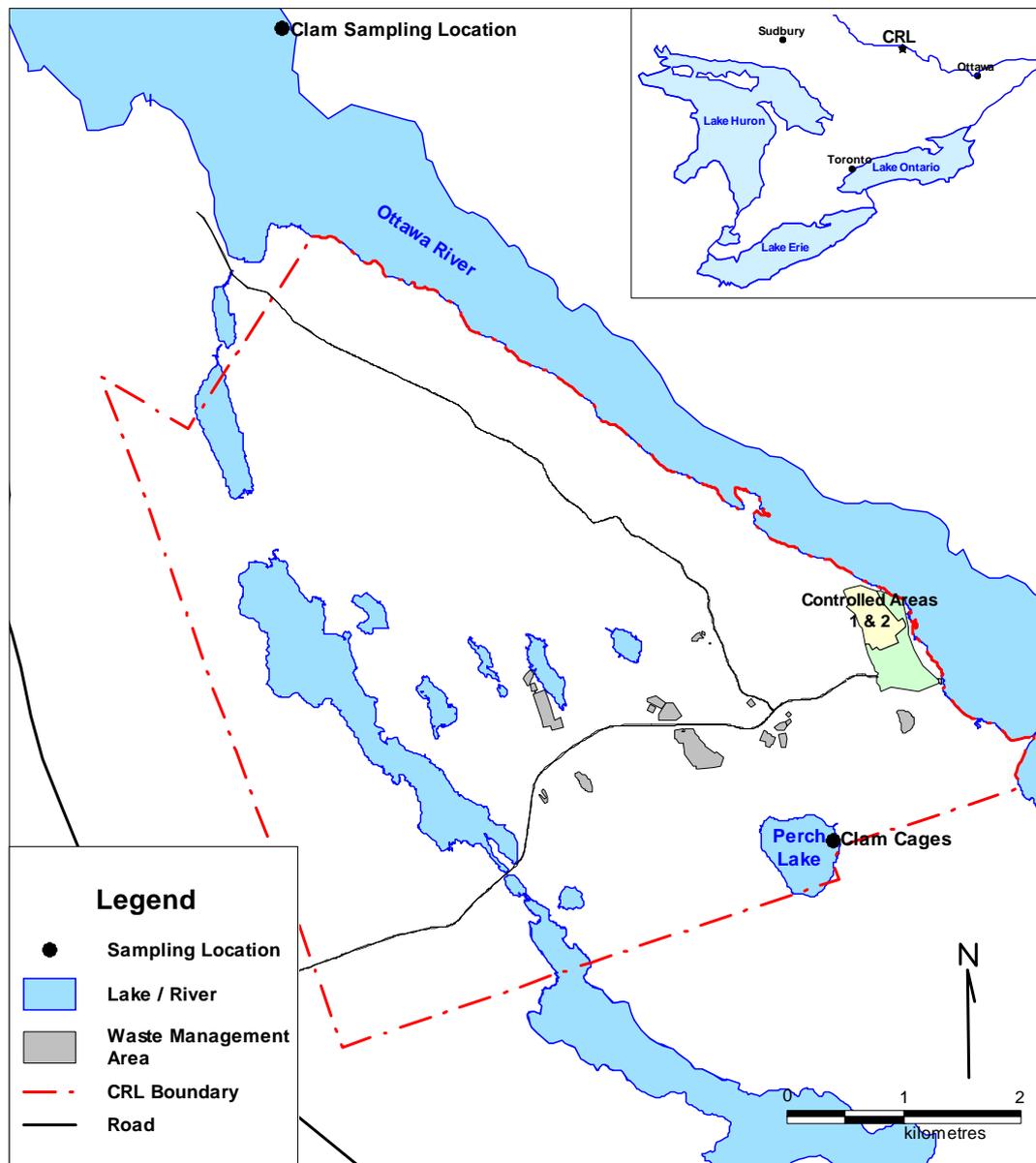


Figure 1: Map depicting the location of the reference site in the Ottawa River where freshwater mussels (*Elliptio complanata*) were collected, relative to the site of mussel transplantation in Perch Lake on AECL’s Chalk River Laboratories site.

Perch Lake Air and Water Temperatures Summer 2004

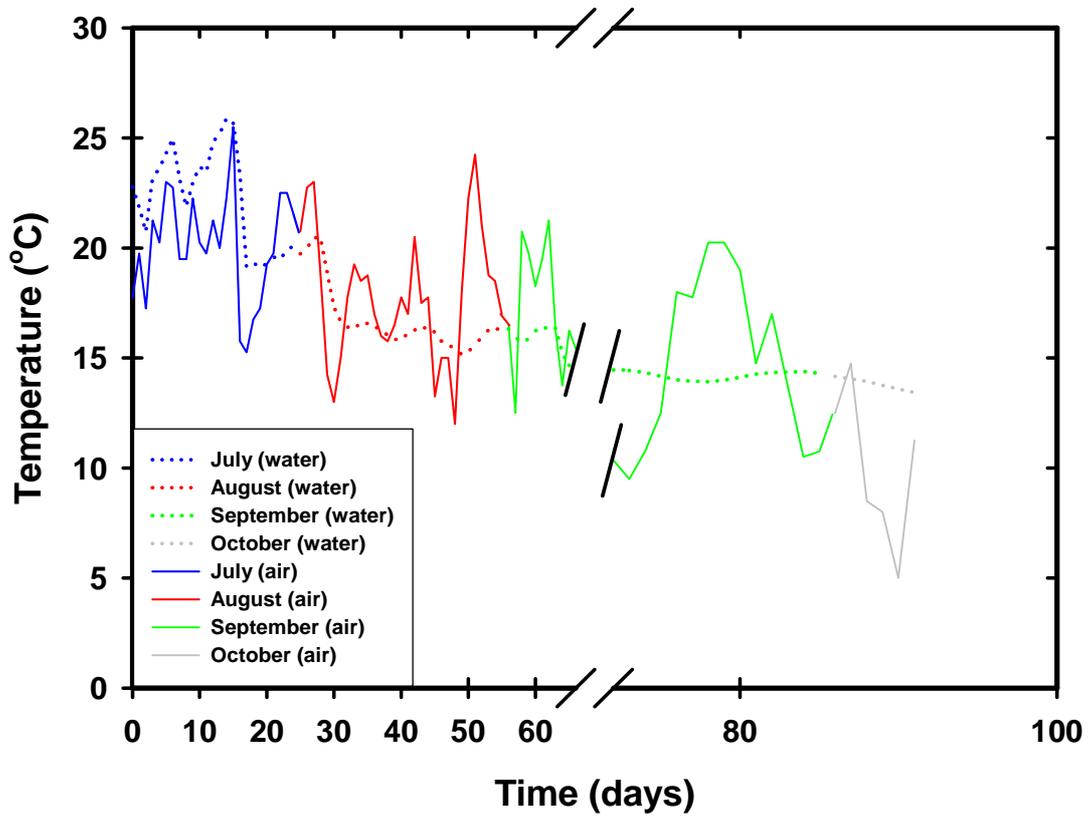


Figure 2: Perch Lake surface water temperatures relative to air temperatures. Temperature measurements were not available over the period between 11 and 17 September 2004.

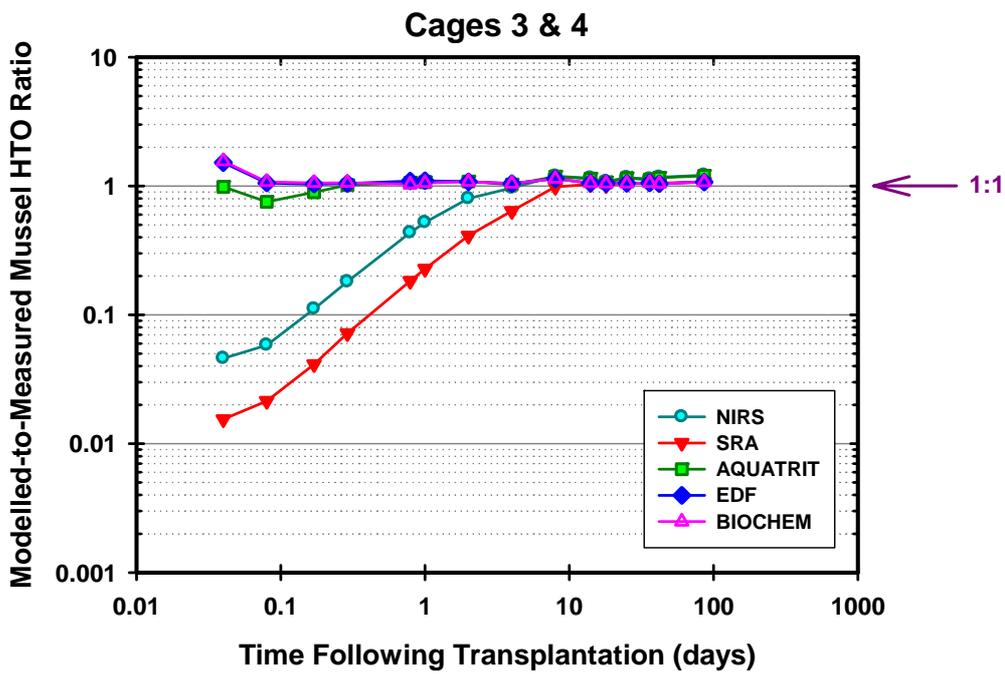
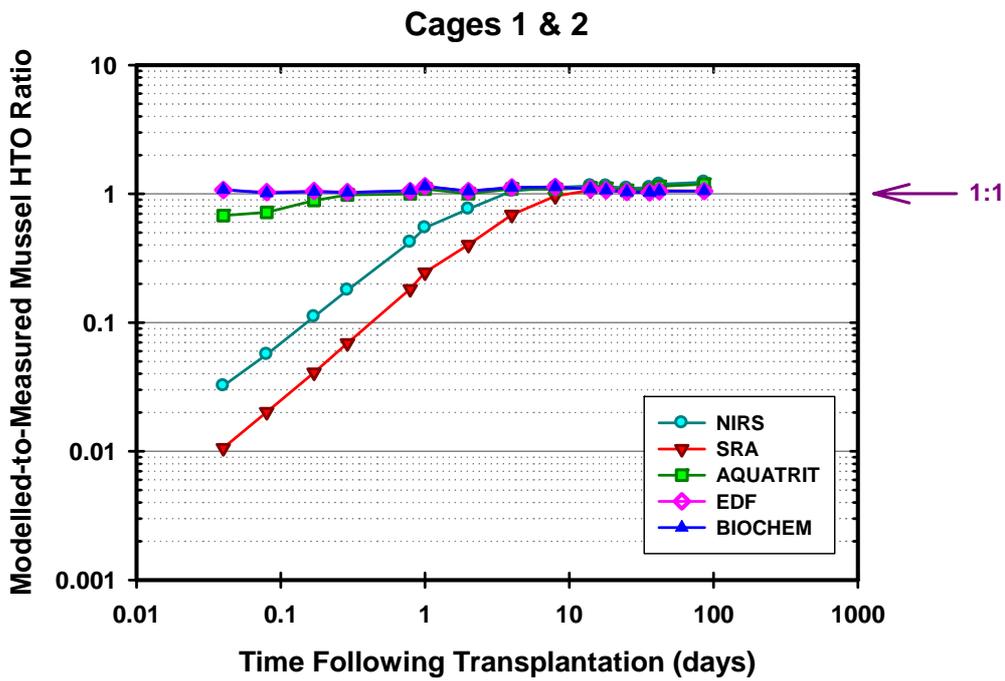


Figure 3: Inter-model comparison of modelled-to-measured HTO concentration in soft tissues of transplanted mussels.

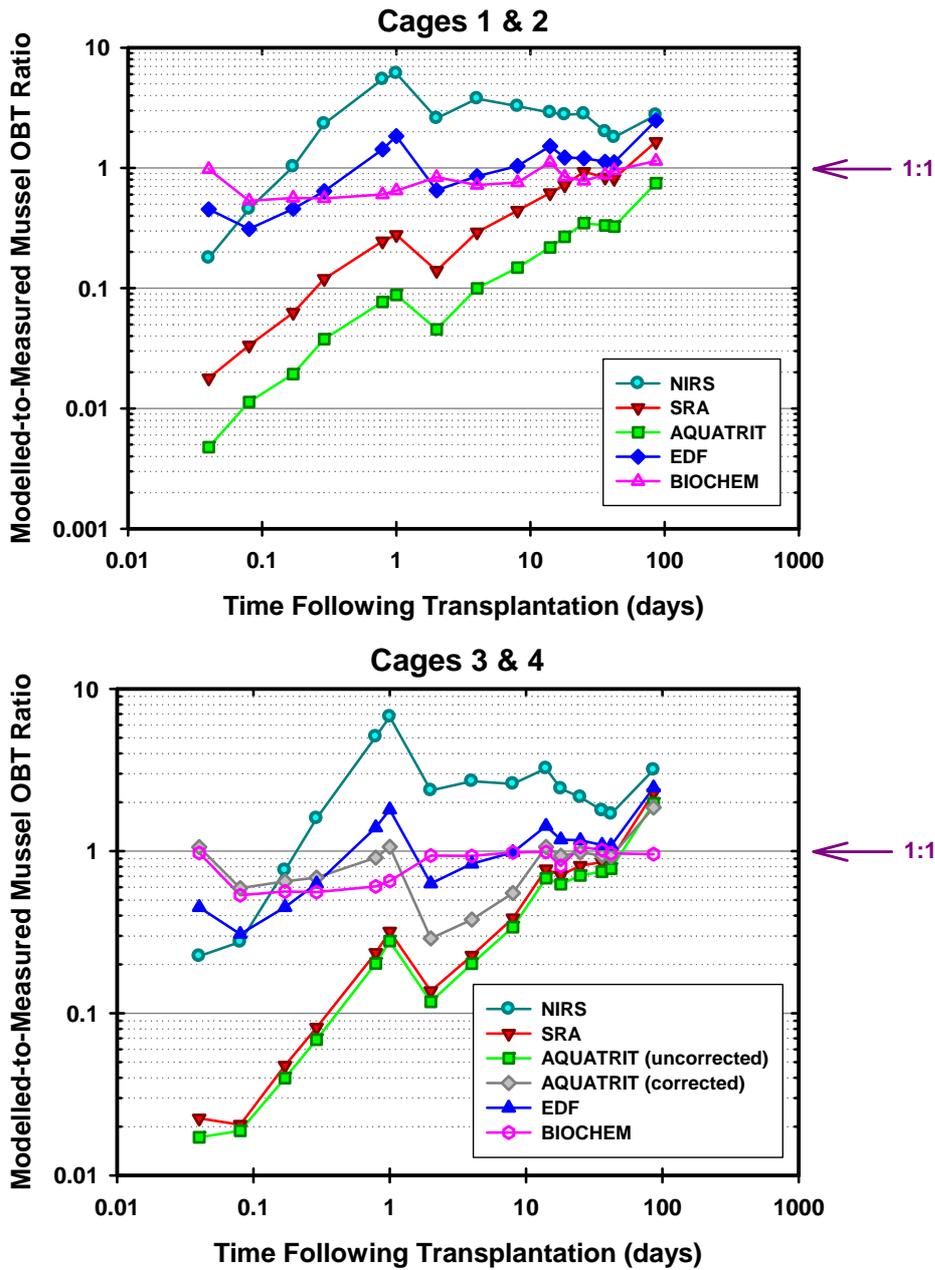


Figure 4: Inter-model comparison of modelled-to-measured OB concentration in soft tissues of transplanted mussels. Uncorrected values predicted by the AQUATRIT model do not account for elevated tritium concentrations in mussel dietary items in Perch Lake, whereas corrected values assume that mussels are feeding on food with tritium levels that are at steady state with Perch Lake HTO levels.

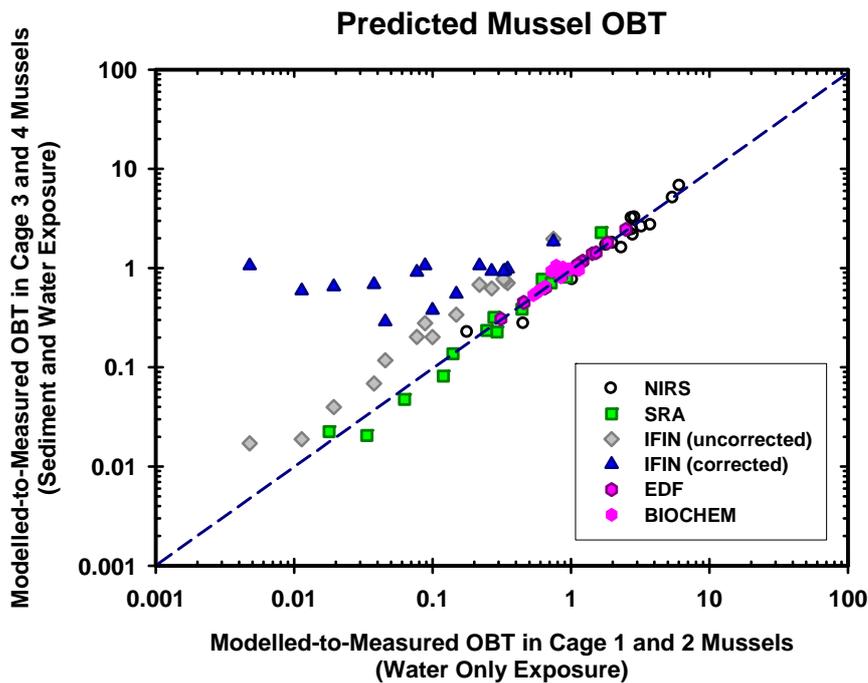
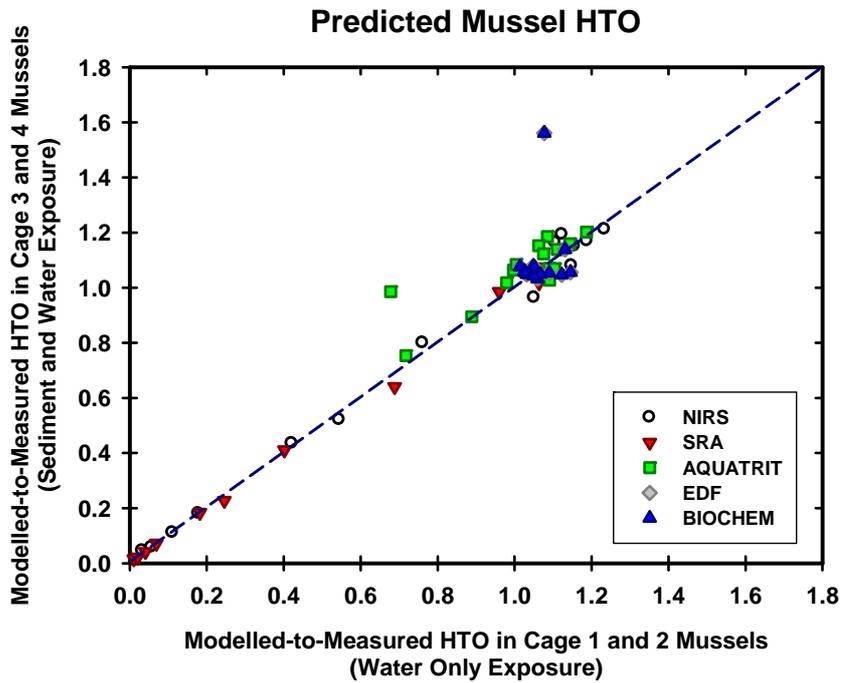


Figure 5: Inter-model comparison of modelled-to-measured HTO and OBT concentrations for mussels in Cages 1 and 2 relative to those for mussels in Cages 3 and 4. Cage 1 and 2 mussels received tritium exposure from the water pathway only. Cage 3 and 4 mussels received tritium exposure from both the sediment and water pathways.

Table 1: Summary of mean monthly Perch Lake surface water and local air temperatures collected over the course of the Perch Lake mussel transplantation study between July and early October 2004.

Month	Mean \pm Standard Error [n] (Minimum to Maximum)		Mean Surface Water-to-Air Ratio	Comments
	Perch Lake Surface Water Temperature ($^{\circ}$ C)	Local Air Temperature ($^{\circ}$ C)		
July	22.3 \pm 0.25 [25] (19.0 to 25.9)	20.1 \pm 0.27 [25] (15.3 to 25.5)	1.11	Represents sampling conducted over the period between July 7 th and 31 st .
August	16.7 \pm 0.16 [31] (15.2 to 20.5)	17.8 \pm 0.33 [31] (12.0 to 24.3)	0.94	Represents sampling conducted over the course of the entire month.
September	14.9 \pm 0.10 [23] (13.9 to 16.4)	16.1 \pm 0.40 [23] (9.5 to 21.3)	0.93	Represents sampling conducted over the month, with the exception of September 11 th to 17 th during which the data were lost.
October	13.8 \pm 0.03 [6] (13.4 to 14.2)	10.0 \pm 0.38 [6] (5.0 to 14.8)	1.38	Represents sampling conducted over the period between October 1 st and 6 th .

Table 2: Summary of weight and length measurements of freshwater mussel specimens at the start of the transplantation study relative to the weight and length at the time of mussel harvest.

Cell No.	Cage No.	<i>Mussel Measurements (Time 0)</i>				Fresh Weight at Harvest Time (g)	Fresh Weight at Harvest-to-Initial Fresh Weight Ratio
		Fresh Weight (g)	Shell Length (mm)	Shell Width (mm)	Shell Height (mm)		
A1	Cage No. 1	64.40	96	46	24	59.49	0.92
	Cage No. 2	60.03	92	49	23	59.46	0.99
	Cage No. 3	100.77	111	58	28	99.50	0.99
	Cage No. 4	78.33	98	49	24	78.89	1.01
A2	Cage No. 1	95.19	98	54	28	91.48	0.96
	Cage No. 2	57.35	92	45	21	65.10	1.14
	Cage No. 3	74.09	96	51	27	71.71	0.97
	Cage No. 4	64.90	95	49	25	64.36	0.99
A3	Cage No. 1	62.94	90	48	25	58.50	0.93
	Cage No. 2	68.62	93	46	26	65.10	0.95
	Cage No. 3	122.57	109	57	33	120.52	0.98
	Cage No. 4	97.13	103	53	27	95.99	0.99
A4	Cage No. 1	83.50	103	49	27	81.06	0.97
	Cage No. 2	61.38	90	45	24	61.58	1.00
	Cage No. 3	62.44	94	46	26	59.449	0.95
	Cage No. 4	60.93	94	45	24	61.23	1.00
A5	Cage No. 1	79.23	99	50	26	76.46	0.97
	Cage No. 2	91.42	105	51	30	90.00	0.98
	Cage No. 3	85.65	103	50	28	85.2	0.99
	Cage No. 4	90.77	105	53	28	90.05	0.99
A6	Cage No. 1	102.05	102	56	27	94.02	0.92
	Cage No. 2	58.94	93	47	23	59.07	1.00
	Cage No. 3	87.57	104	56	28	86.55	0.99
	Cage No. 4	77.47	103	51	25	74.06	0.96
A7	Cage No. 1	69.89	95	49	24	68.55	0.98
	Cage No. 2	74.51	96	52	26	71.98	0.97
	Cage No. 3	56.50	92	52	20	56.006	0.99
	Cage No. 4	100.44	109	57	29	100.61	1.00

Cell No.	Cage No.	<i>Mussel Measurements (Time 0)</i>				Fresh Weight at Harvest Time (g)	Fresh Weight at Harvest-to-Initial Fresh Weight Ratio
		Fresh Weight (g)	Shell Length (mm)	Shell Width (mm)	Shell Height (mm)		
A8	Cage No. 1	83.58	96	51	27	82.54	0.99
	Cage No. 2	72.89	94	50	26	72.13	0.99
	Cage No. 3	61.72	92	46	25	60.38	0.98
	Cage No. 4	70.48	90	51	25	69.21	0.98
B1	Cage No. 1	73.07	96	46	27	71.30	0.98
	Cage No. 2	90.96	100	54	30	89.41	0.98
	Cage No. 3	82.79	101	53	26	82.79	1.00
	Cage No. 4	69.16	90	49	25	69.7	1.01
B2	Cage No. 1	75.31	95	48	26	73.67	0.98
	Cage No. 2	98.10	105	54	32	97.78	1.00
	Cage No. 3	86.19	107	55	25	84.85	0.98
	Cage No. 4	117.87	109	59	31	116.1	0.98
B3	Cage No. 1	77.75	95	51	27	76.64	0.99
	Cage No. 2	79.26	95	52	29	78.41	0.99
	Cage No. 3	75.66	99	53	27	75.86	1.00
	Cage No. 4	73.90	100	51	26	72.96	0.99
B4	Cage No. 1	94.55	104	54	28	92.13	0.97
	Cage No. 2	73.14	94	51	27	71.56	0.98
	Cage No. 3	72.95	98	51	26	72.2	0.99
	Cage No. 4	85.76	102	52	26	84.92	0.99
B5	Cage No. 1	66.31	94	49	26	65.89	0.99
	Cage No. 2	70.63	94	53	27	68.25	0.97
	Cage No. 3	74.28	103	51	27	72.62	0.98
	Cage No. 4	73.64	100	49	24	75.6	1.03
B6	Cage No. 1	98.34	106	56	27	96.76	0.98
	Cage No. 2	62.84	90	51	35	61.23	0.97
	Cage No. 3	101.33	110	54	30	100.94	1.00
	Cage No. 4	83.43	104	52	25	82.43	0.99
B7	Cage No. 1	70.41	95	49	26	67.7	0.96
	Cage No. 2	65.22	96	47	27	64.2	0.98
	Cage No. 3	91.92	100	54	28	91.7	1.00
	Cage No. 4	77.91	93	50	26	78.04	1.00

Cell No.	Cage No.	<i>Mussel Measurements (Time 0)</i>				Fresh Weight at Harvest Time (g)	Fresh Weight at Harvest-to-Initial Fresh Weight Ratio
		Fresh Weight (g)	Shell Length (mm)	Shell Width (mm)	Shell Height (mm)		
B8	Cage No. 1	70.29	103	47	22	69.64	0.99
	Cage No. 2	70.75	90	51	29	70.73	1.00
	Cage No. 3	74.20	99	50	28	74.34	1.00
	Cage No. 4	78.51	98	49	26	77.8	0.99
C1	Cage No. 1	67.95	97	47	25	67.76	1.00
	Cage No. 2	73.15	100	46	26	72.02	0.98
	Cage No. 3	102.75	108	53	31	98.87	0.96
	Cage No. 4	69.39	95	47	27	67.49	0.97
C2	Cage No. 1	80.67	104	54	25	80.97	1.00
	Cage No. 2	62.98	94	58	26	61.20	0.97
	Cage No. 3	68.65	97	47	24	68.64	1.00
	Cage No. 4	84.76	100	50	27	84.32	0.99
C3	Cage No. 1	57.44	93	45	23	57.177	1.00
	Cage No. 2	77.36	100	55	26	74.69	0.97
	Cage No. 3	71.25	99	48	27	69.43	0.97
	Cage No. 4	57.55	95	47	21	57.505	1.00
C4	Cage No. 1	79.36	104	52	25	77.4	0.98
	Cage No. 2	79.90	98	48	28	80.56	1.01
	Cage No. 3	83.91	105	53	29	83.87	1.00
	Cage No. 4	94.57	105	55	26	94.43	1.00
C5	Cage No. 1	73.39	96	50	25	70.86	0.97
	Cage No. 2	63.48	95	52	23	62.24	0.98
	Cage No. 3	84.51	103	51	29	84.16	1.00
	Cage No. 4	67.19	102	50	22	65.26	0.97
C6	Cage No. 1	86.02	99	49	30	85.12	0.99
	Cage No. 2	81.52	100	52	26	80.2	0.98
	Cage No. 3	78.38	104	51	26	76.1	0.97
	Cage No. 4	94.18	105	50	29	93.12	0.99
C7	Cage No. 1	83.06	101	52	26	82.55	0.99
	Cage No. 2	82.38	102	59	30	81.61	0.99
	Cage No. 3	70.38	98	47	27	67.64	0.96
	Cage No. 4	78.38	100	51	27	76.63	0.98

Cell No.	Cage No.	<i>Mussel Measurements (Time 0)</i>				Fresh Weight at Harvest Time (g)	Fresh Weight at Harvest-to-Initial Fresh Weight Ratio
		Fresh Weight (g)	Shell Length (mm)	Shell Width (mm)	Shell Height (mm)		
C8	Cage No. 1	74.35	101	46	26	73.06	0.98
	Cage No. 2	119.84	109	57	33	115.86	0.97
	Cage No. 3	81.21	104	54	27	81.16	1.00
	Cage No. 4	80.26	98	50	27	81.31	1.01
D1	Cage No. 1	101.37	103	58	27	97.39	0.96
	Cage No. 2	113.44	110	56	30	112.9	1.00
	Cage No. 3	117.32	106	60	30	116.93	1.00
	Cage No. 4	70.64	95	50	24	69.49	0.98
D2	Cage No. 1	101.61	101	55	29	99.5	0.98
	Cage No. 2	96.75	104	56	30	94.91	0.98
	Cage No. 3	78.61	102	55	28	78.68	1.00
	Cage No. 4	80.66	99	52	26	81.01	1.00
D3	Cage No. 1	83.65	102	50	25	82.31	0.98
	Cage No. 2	97.71	101	59	30	95.74	0.98
	Cage No. 3	77.04	100	50	26	77.41	1.00
	Cage No. 4	81.01	101	51	25	81.39	1.00
D4	Cage No. 1	68.54	96	49	29	66.12	0.96
	Cage No. 2	116.83	110	51	33	113.79	0.97
	Cage No. 3	71.61	94	50	26	70.53	0.98
	Cage No. 4	82.94	104	51	26	80.45	0.97
D5	Cage No. 1	69.29	95	49	26	69.18	1.00
	Cage No. 2	68.78	93	53	25	67.99	0.99
	Cage No. 3	103.58	109	55	30	100.6	0.97
	Cage No. 4	78.11	99	51	25	77.81	1.00
D6	Cage No. 1	78.06	99	49	27	76.21	0.98
	Cage No. 2	98.91	104	50	30	97.65	0.99
	Cage No. 3	74.73	93	53	24	75.21	1.01
	Cage No. 4	86.86	105	51	26	84.54	0.97
D7	Cage No. 1	74.73	99	50	25	72.37	0.97
	Cage No. 2	56.23	94	50	24	55.53	0.99
	Cage No. 3	91.28	99	54	29	90.39	0.99
	Cage No. 4	74.43	100	51	26	74.86	1.01

Cell No.	Cage No.	<i>Mussel Measurements (Time 0)</i>				Fresh Weight at Harvest Time (g)	Fresh Weight at Harvest-to-Initial Fresh Weight Ratio
		Fresh Weight (g)	Shell Length (mm)	Shell Width (mm)	Shell Height (mm)		
D8	Cage No. 1	68.01	95	45	25	67.69	1.00
	Cage No. 2	78.77	94	52	28	76.85	0.98
	Cage No. 3	76.94	96	51	24	77.54	1.01
	Cage No. 4	67.74	91	45	26	65.99	0.97
E1	Cage No. 1	70.48	101	50	23	70.61	1.00
	Cage No. 2	94.40	100	58	30	93.63	0.99
	Cage No. 3	75.84	100	51	27	74.22	0.98
	Cage No. 4	56.26	93	46	24	56.267	1.00
E2	Cage No. 1	83.36	104	53	26	80.08	0.96
	Cage No. 2	93.48	100	52	30	89.86	0.96
	Cage No. 3	85.21	96	51	29	83.04	0.97
	Cage No. 4	74.88	94	52	25	74.19	0.99
E3	Cage No. 1	75.97	96	50	27	75	0.99
	Cage No. 2	87.74	104	53	29	85.62	0.98
	Cage No. 3	108.61	101	54	34	105.06	0.97
	Cage No. 4	67.46	100	50	21	67.53	1.00
E4	Cage No. 1	94.02	106	55	32	91.5	0.97
	Cage No. 2	84.80	101	54	29	83.68	0.99
	Cage No. 3	121.49	106	58	32	120.72	0.99
	Cage No. 4	82.10	91	50	28	82.6	1.01
E5	Cage No. 1	68.08	97	48	25	66.73	0.98
	Cage No. 2	78.27	98	50	29	75.46	0.96
	Cage No. 3	71.57	98	50	25	70.37	0.98
	Cage No. 4	93.52	106	54	26	94.4	1.01
E6	Cage No. 1	94.80	99	50	29	88.33	0.93
	Cage No. 2	59.17	90	48	24	58.416	0.99
	Cage No. 3	67.72	94	49	26	66.59	0.98
	Cage No. 4	79.62	100	54	24	77.65	0.98
E7	Cage No. 1	76.23	96	54	25	73.29	0.96
	Cage No. 2	90.52	102	57	29	88	0.97
	Cage No. 3	67.71	98	46	25	68.7	1.01
	Cage No. 4	68.97	94	47	26	67.4	0.98

Cell No.	Cage No.	<i>Mussel Measurements (Time 0)</i>				Fresh Weight at Harvest Time (g)	Fresh Weight at Harvest-to-Initial Fresh Weight Ratio
		Fresh Weight (g)	Shell Length (mm)	Shell Width (mm)	Shell Height (mm)		
E8	Cage No. 1	72.53	96	48	26	71.23	0.98
	Cage No. 2	84.61	102	53	28	81.36	0.96
	Cage No. 3	91.71	100	54	28	89.95	0.98
	Cage No. 4	64.47	94	48	25	63.28	0.98
F1	Cage No. 1	82.47	100	56	25	78.14	0.95
	Cage No. 2	106.65	108	55	31	105.95	0.99
	Cage No. 3	118.56	106	56	35	115.79	0.98
	Cage No. 4	72.55	102	50	23	70.81	0.98
F2	Cage No. 1	71.93	92	45	26	69.54	0.97
	Cage No. 2	83.38	100	53	30	81.72	0.98
	Cage No. 3	93.37	108	55	27	87.03	0.93
	Cage No. 4	75.37	97	51	24	74.34	0.99
F3	Cage No. 1	64.14	95	46	25	61.8	0.96
	Cage No. 2	70.93	99	49	26	70.59	1.00
	Cage No. 3	84.16	98	54	28	82.23	0.98
	Cage No. 4	77.31	100	50	27	76.95	1.00
F4	Cage No. 1	64.66	90	43	27	63.85	0.99
	Cage No. 2	62.23	94	52	25	60.1	0.97
	Cage No. 3	52.74	95	44	22	51.241	0.97
	Cage No. 4	56.74	92	46	24	56.236	0.99
F5	Cage No. 1	57.42	96	46	20	56.668	0.99
	Cage No. 2	66.86	94	52	27	66.36	0.99
	Cage No. 3	86.67	96	56	27	86.99	1.00
	Cage No. 4	61.29	93	48	23	61.02	1.00
F6	Cage No. 1	62.56	91	45	24	61.05	0.98
	Cage No. 2	81.23	96	55	28	81.23	1.00
	Cage No. 3	87.95	99	51	27	85.63	0.97
	Cage No. 4	85.34	101	50	26	85.03	1.00
F7	Cage No. 1	77.95	96	50	25	75.62	0.97
	Cage No. 2	86.17	100	50	30	83.18	0.97
	Cage No. 3	78.95	101	50	25	75.58	0.96
	Cage No. 4	88.66	105	51	25	86.43	0.97

Cell No.	Cage No.	<i>Mussel Measurements (Time 0)</i>				Fresh Weight at Harvest Time (g)	Fresh Weight at Harvest-to-Initial Fresh Weight Ratio
		Fresh Weight (g)	Shell Length (mm)	Shell Width (mm)	Shell Height (mm)		
F8	Cage No. 1	103.22	102	52	32	100.25	0.97
	Cage No. 2	80.08	98	50	27	78.4	0.98
	Cage No. 3	78.25	96	56	27	76.98	0.98
	Cage No. 4	79.17	96	50	26	78.81	1.00
G1	Cage No. 1	93.02	100	50	29	90.27	0.97
	Cage No. 2	84.70	102	56	28	82.26	0.97
	Cage No. 3	75.21	92	49	29	74.76	0.99
	Cage No. 4	97.28	101	53	28	96.13	0.99
G2	Cage No. 1	87.85	100	51	27	85.82	0.98
	Cage No. 2	81.72	96	52	29	80.41	0.98
	Cage No. 3	88.85	100	50	29	84.76	0.95
	Cage No. 4	68.91	100	49	24	68.65	1.00
G3	Cage No. 1	81.58	98	52	27	79.63	0.98
	Cage No. 2	92.11	101	59	28	92.06	1.00
	Cage No. 3	73.52	95	48	27	71.62	0.97
	Cage No. 4	57.64	95	43	25	57.56	1.00
G4	Cage No. 1	78.90	103	49	25	75.92	0.96
	Cage No. 2	76.98	101	49	28	75.28	0.98
	Cage No. 3	96.64	104	51	30	92.47	0.96
	Cage No. 4	65.54	95	49	25	65.23	1.00
G5	Cage No. 1	81.23	98	50	26	78.49	0.97
	Cage No. 2	85.68	103	54	27	85.27	1.00
	Cage No. 3	87.76	99	52	26	87.03	0.99
	Cage No. 4	59.86	93	46	24	59.814	1.00
G6	Cage No. 1	75.92	104	50	26	72.72	0.96
	Cage No. 2	69.04	93	49	24	67.95	0.98
	Cage No. 3	87.04	94	51	26	85.87	0.99
	Cage No. 4	78.69	101	48	26	78.23	0.99
G7	Cage No. 1	82.61	99	51	26	78.7	0.95
	Cage No. 2	102.42	109	58	28	94.75	0.93
	Cage No. 3	90.70	105	52	29	87.91	0.97
	Cage No. 4	77.30	95	50	28	77.06	1.00

Cell No.	Cage No.	<i>Mussel Measurements (Time 0)</i>				Fresh Weight at Harvest Time (g)	Fresh Weight at Harvest-to-Initial Fresh Weight Ratio
		Fresh Weight (g)	Shell Length (mm)	Shell Width (mm)	Shell Height (mm)		
G8	Cage No. 1	101.38	101	55	30	95.56	0.94
	Cage No. 2	111.92	105	54	32	108.58	0.97
	Cage No. 3	77.33	93	51	27	75.57	0.98
	Cage No. 4	71.08	95	49	26	71.36	1.00
H1	Cage No. 1	99.11	99	51	29	97.53	0.98
	Cage No. 2	58.79	95	49	23	56.92	0.97
	Cage No. 3	78.30	96	50	27	75.04	0.96
	Cage No. 4	88.84	99	52	28	89.2	1.00
H2	Cage No. 1	102.84	106	58	29	99.02	0.96
	Cage No. 2	76.84	100	52	27	73.43	0.96
	Cage No. 3	73.16	101	51	22	70.6	0.97
	Cage No. 4	70.65	97	48	25	68.19	0.97
H3	Cage No. 1	89.06	105	54	27	84.9	0.95
	Cage No. 2	91.36	105	57	27	89.78	0.98
	Cage No. 3	76.54	97	50	27	74.96	0.98
	Cage No. 4	62.94	91	48	25	60.85	0.97
H4	Cage No. 1	71.87	92	48	24	71.17	0.99
	Cage No. 2	97.37	104	60	30	91.01	0.93
	Cage No. 3	78.72	94	49	27	77	0.98
	Cage No. 4	78.80	100	50	26	77.18	0.98
H5	Cage No. 1	99.63	107	59	29	98	0.98
	Cage No. 2	82.38	102	54	29	79.17	0.96
	Cage No. 3	93.95	105	54	28	92.17	0.98
	Cage No. 4	59.08	91	46	23	58.193	0.98
H6	Cage No. 1	86.78	101	50	27	81.68	0.94
	Cage No. 2	79.57	96	51	30	75.8	0.95
	Cage No. 3	79.56	101	51	25	77.41	0.97
	Cage No. 4	75.75	98	51	25	74.1	0.98
H7	Cage No. 1	87.75	100	51	28	85.58	0.98
	Cage No. 2	92.28	99	55	30	89.54	0.97
	Cage No. 3	87.52	102	51	26	85.56	0.98
	Cage No. 4	76.51	94	50	25	74.86	0.98

Cell No.	Cage No.	<i>Mussel Measurements (Time 0)</i>				Fresh Weight at Harvest Time (g)	Fresh Weight at Harvest-to-Initial Fresh Weight Ratio
		Fresh Weight (g)	Shell Length (mm)	Shell Width (mm)	Shell Height (mm)		
H8	Cage No. 1	99.67	107	56	27	96.14	0.96
	Cage No. 2	67.62	96	49	26	66.23	0.98
	Cage No. 3	73.50	101	48	25	71.24	0.97
	Cage No. 4	65.86	93	49	25	64.06	0.97

Table 3: Summary of tritium concentrations in Perch Lake surface waters (provided as model input data) (from Yankovich and Kim, 2005).

Time After Mussel Transplantation	Cage Numbers	Arithmetic Mean HTO in Water (Bq/L)	Standard Error	n	Minimum	Maximum
0 hours (all cages)	Cages 1 and 2	4,796	26.9	6	4,689	4,880
	Cages 3 and 4	4,698	27.7	6	4,636	4,799
1 hour (all cages)	Cages 1 and 2	4,766	23.8	6	4,685	4,830
	Cages 3 and 4	4,749	30.3	6	4,646	4,844
2 hours (all cages)	Cages 1 and 2	4,664	31.7	6	4,575	4,795
	Cages 3 and 4	4,713	19.7	6	4,638	4,766
4 hours (all cages)	Cages 1 and 2	4,681	22.5	6	4,598	4,747
	Cages 3 and 4	4,724	26.9	6	4,660	4,835
7 hours (all cages)	Cages 1 and 2	4,712	29.9	6	4,611	4,804
	Cages 3 and 4	4,686	29.9	6	4,566	4,769
19 hours (all cages)	Cages 1 and 2	4,783	19.1	6	4,716	4,840
	Cages 3 and 4	4,368	18.9	6	4,329	4,456
24 hours (all cages)	Cages 1 and 2	4,731	25.5	6	4,677	4,832
	Cages 3 and 4	4,441	23.5	6	4,371	4,522
48 hours (all cages)	Cages 1 and 2	4,698	27.7	6	4,636	4,799
	Cages 3 and 4	4,476	49.9	6	4,329	4,648
96 hours (all cages)	Cages 1 and 2	4,629	15.9	6	4,597	4,699
	Cages 3 and 4	4,583	30.9	6	4,526	4,722
8 days (all cages)	Cages 1 and 2	4,690	15.2	6	4,634	4,749
	Cages 3 and 4	4,323	33.2	6	4,200	4,431
14 days (all cages)	Cages 1 and 2	4,416	33.4	6	4,298	4,533
	Cages 3 and 4	4,163	12.8	6	4,128	4,212
18 days (Cages 1 & 2)	Cages 1 and 2	4,352	21.3	6	4,276	4,438
19 days (Cages 3 & 4)	Cages 3 and 4	4,417	14.6	6	4,374	4,470
25 days (Cages 1 & 2)	Cages 1 and 2	4,367	19.3	6	4,299	4,420
27 days (Cages 3 & 4)	Cages 3 and 4	4,093	24.5	6	3,985	4,143
36 days (Cages 1 & 2)	Cages 1 and 2	4,298	33.2	6	4,191	4,393
35 days (Cages 3 & 4)	Cages 3 and 4	4,231	29.3	6	4,150	4,328
42 days (Cages 1 & 2)	Cages 1 and 2	4,130	16.8	6	4,079	4,182
41 days (Cages 3 & 4)	Cages 3 and 4	4,048	20.6	6	3,977	4,094
^a 77 days	Perch Lake	4,065	15.3	3	4,038	4,091
86 days (Cages 1 & 2)	Cages 1 and 2	3,985	35.4	4	3,930	4,088
84 days (Cages 3 & 4)	Cages 3 and 4	4,025	23.9	4	3,955	4,062

Measurement error for HTO was <1%.

^a Triplicate water samples were collected in the area where the plankton samples were taken. Water data are likely representative of a well-mixed condition in the lake.

Table 4: Summary of tritium concentrations in Perch Lake sediments (provided as model input data) (from Yankovich and Kim, 2005).

Time After Mussel Transplantation	Cage Numbers	Arithmetic Mean HTO in Sediments (Bq/L) ± Standard Error [n]	OBT ± Counting Error
0 hours (all cages)	Cages 1 and 2	n.a.	n.a.
	Cages 3 and 4	4,303 ± 7.0 [2]	1,020 ± 26
1 hour (all cages)	Cages 1 and 2	3,944 ± 17.5 [2]	994 ± 23
	Cages 3 and 4	n.a.	n.a.
2 hours (all cages)	Cages 1 and 2	n.a.	n.a.
	Cages 3 and 4	n.a.	n.a.
4 hours (all cages)	Cages 1 and 2	n.a.	n.a.
	Cages 3 and 4	n.a.	n.a.
7 hours (all cages)	Cages 1 and 2	n.a.	n.a.
	Cages 3 and 4	n.a.	n.a.
19 hours (all cages)	Cages 1 and 2	4,020 ± 5.0 [2]	700 ± 7
	Cages 3 and 4	3,828 ± 26.0 [2]	1,248 ± 50
24 hours (all cages)	Cages 1 and 2	n.a.	n.a.
	Cages 3 and 4	n.a.	n.a.
48 hours (all cages)	Cages 1 and 2	n.a.	n.a.
	Cages 3 and 4	n.a.	n.a.
96 hours (all cages)	Cages 1 and 2	n.a.	n.a.
	Cages 3 and 4	n.a.	n.a.
8 days (all cages)	Cages 1 and 2	3,956 ± 37.0 [2]	571 ± 9
	Cages 3 and 4	3,820 ± 25.0 [2]	1,403 ± 66
14 days (all cages)	Cages 1 and 2	n.a.	n.a.
	Cages 3 and 4	n.a.	n.a.
18 days (Cages 1 & 2)	Cages 1 and 2	n.a.	n.a.
19 days (Cages 3 & 4)	Cages 3 and 4	n.a.	n.a.
25 days (Cages 1 & 2)	Cages 1 and 2	n.a.	n.a.
27 days (Cages 3 & 4)	Cages 3 and 4	3,885 ± 9.0 [2]	1,159 ± 33
36 days (Cages 1 & 2)	Cages 1 and 2	3,830 ± 27.5 [2]	704 ± 17
35 days (Cages 3 & 4)	Cages 3 and 4	n.a.	n.a.
42 days (Cages 1 & 2)	Cages 1 and 2	n.a.	n.a.
41 days (Cages 3 & 4)	Cages 3 and 4	n.a.	n.a.
86 days (Cages 1 & 2)	Cages 1 and 2	n.a.	n.a.
84 days (Cages 3 & 4)	Cages 3 and 4	3,557 ± 28.3 [2]	1,829 ± 28 (Cage 3)
			1,981 ± 57 (Cage 4)
All Data	Cages 1 and 2	3,937 ± 28 [8]	742 ± 89 [4]
	Cages 3 and 4	3,879 ± 91 [10]	1,440 ± 157 [6]

n.a. – data not available, since only a subset of sediment samples were analyzed.

Table 5: Summary of HTO and OBT concentrations measured in mussel soft tissues following transplantation from a background location on the Ottawa River to Perch Lake.

Time After Mussel Transplantation	Time (days)	Cage Numbers	Measured HTO (Bq/L) ± Standard Error	Measured OBT (Bq/L) ± Counting Error
0 hours	0	not applicable	< 10	45
1 hour	0.04	Cages 1 and 2	4,425 ± 5.8	168 ± 1
		Cages 3 and 4	3,042 ± 22	134 ± 7.5
2 hours	0.08	Cages 1 and 2	4,599 ± 23	150 ± 7
		Cages 3 and 4	4,382 ± 13	244 ± 11.5
4 hours	0.17	Cages 1 and 2	4,501 ± 27	176 ± 19
		Cages 3 and 4	4,472 ± 25	231 ± 30
7 hours	0.29	Cages 1 and 2	4,594 ± 22	159 ± 1
		Cages 3 and 4	4,422 ± 14	233 ± 14
19 hours	0.79	Cages 1 and 2	4,515 ± 9.8	208 ± 16
		Cages 3 and 4	4,231 ± 9.2	217 ± 10.5
24 hours	1	Cages 1 and 2	4,131 ± 44	227 ± 7
		Cages 3 and 4	4,205 ± 27	201 ± 9.5
48 hours	2	Cages 1 and 2	4,481 ± 15	879 ± 29
		Cages 3 and 4	4,151 ± 9.8	934 ± 138
96 hours	4	Cages 1 and 2	4,126 ± 19	802 ± 89
		Cages 3 and 4	4,379 ± 17	1,090 ± 36.5
8 days	8	Cages 1 and 2	4,147 ± 8.1	1,013 ± 55
		Cages 3 and 4	3,796 ± 12	1,236 ± 268.5
14 days	14	Cages 1 and 2	4,050 ± 11	1,147 ± 42
		Cages 3 and 4	3,951 ± 17	999 ± 114
18 days	18	Cages 1 and 2	4,078 ± 20	1,198 ± 230
19 days	19	Cages 3 and 4	4,209 ± 26	1,330 ± 11.5
25 days	25	Cages 1 and 2	4,234 ± 31	1,179 ± 102
27 days	27	Cages 3 and 4	3,904 ± 18	1,498 ± 80
36 days	36	Cages 1 and 2	4,185 ± 13	1,657 ± 186
35 days	35	Cages 3 and 4	4,008 ± 13	1,809 ± 54
42 days	42	Cages 1 and 2	3,936 ± 19	1,844 ± 132
41 days	41	Cages 3 and 4	3,880 ± 13	1,900 ± 90.5
86 days	86	Cages 1 and 2	3,791 ± 21	1,206 ± 125
84 days	84	Cages 3 and 4	3,745 ± 16	1,016 ± 68.5

Table 6: Models and modellers participating in the Perch Lake uptake scenario.

Model Name	Lead Modeller	Affiliation	Country
NIRS	Kiriko Miyamoto	National Institute of Radiological Sciences (NIRS)	Japan
SRA	Masahiro Saito	Kyoto University Safety Reassurance Academy (SRA)	Japan
AQUATRIT	Dan Galeriu and Anca Melintescu	Institute of Atomic Physics & Nuclear Engineering - Horia Hulubei (IFIN-HH)	Romania
EDF	Francoise Siclet	Electricité de France (EDF)	France
BIOCHEM	Franz Baumgärtner	Technical University Munich, Institute of Radiochemistry	Germany

Table 7: Summary of key model assumptions.

Model Name	Country	Type of Model	No. of Compartments	Model Compartments*	Does Model Account for Water Temperature?	Does Model Assume Dietary Assimilation to Dilute OBT?
NIRS	Japan	Dynamic	3	HTO, OBT-1, OBT-2	No	No
SRA	Japan	Dynamic	3	HTO, OBT-1, OBT-2	No	Yes
AQUATRIT	Romania	Dynamic	2	HTO, OBT	No	Yes
EDF	France	Dynamic	2	HTO, OBT	No	Yes
BIOCHEM	Germany	Steady state	4	HTO, CBT, YBT, XBT	Yes	No

* HTO: free-water tritium; OBT: organically-bound tritium; CBT: carbon-bound tritium; YBT: hydrate-bound tritium; XBT: tritium that is bound to oxygen, nitrogen or sulphur atoms (representing a form of exchangeable OBT).

Table 8: Summary of outcomes of linear regression and Analysis of Covariance (ANCOVA) analyses for modelled-to-measured initial rate of HTO uptake by mussels following transplantation into Perch Lake.

Model	Cage Nos.	Linear Regression Analysis					Analysis of Covariance (ANCOVA)	
		Range Considered in Linear Regression		$\text{LOG} \left(\frac{\text{Predicted [HTO]}_{\text{mussel}}}{\text{Measured [HTO]}_{\text{mussel}}} \right) = m \cdot \text{LOG}(\text{time}) + b$			p-value	Interpretation
		Start Time (days)	End Time (days)	Slope, m	y-intercept, b	r ² -value		
NIRS	1 and 2	0.04	1	0.876	-0.277	99.96%	0.8322	^a Equal slopes
	3 and 4	0.08	1	0.873	-0.278	99.98%		
SRA	1 and 2	0.04	1	0.969	-0.630	99.94%	0.1704	^a Equal slopes
	3 and 4	0.08	1	0.942	-0.643	99.95%		
AQUATRIT	1 and 2	0.04	1	0.145	0.0382	92.4%	0.7388	^a Equal slopes
	3 and 4	0.08	1	0.133	0.0451	88.1%		
EDF	1 and 2	0.08	1	0.0287	0.0398	46.1%	0.7116	^a Equal slopes
	3 and 4	0.08	1	0.0209	0.038	65.4%		
BIOCHEM	1 and 2	0.08	1	0.0369	0.0421	64.9%	0.0288	^b Unequal slopes
	3 and 4	0.08	1	-0.0104	0.0184	56.3%		

^a Equal rates of HTO increase for mussels receiving exposure from sediments and water compared to mussels exposed to water only.

^b HTO uptake rates are predicted to be marginally significantly faster for mussels exposed to HTO via water only compared to those exposed via both water and sediments.

Table 9: Summary of outcomes of linear regression and Analysis of Covariance (ANCOVA) analyses for modelled-to-measured initial rate of OBT formation by mussels following transplantation into Perch Lake.

Model	Cage Nos.	Linear Regression Analysis					Analysis of Covariance (ANCOVA)	
		Range Considered in Linear Regression		$\text{LOG} \left(\frac{\text{Predicted [OBT]}_{\text{mussel}}}{\text{Measured [OBT]}_{\text{mussel}}} \right) = m \cdot \text{LOG}(\text{time}) + b$			p-value	Interpretation
		Start Time (days)	End Time (days)	Slope, m	y-intercept, b	r ² -value		
NIRS	1 and 2	0.04	1	4.48	5.51	90.8%	0.294	^a Equal slopes
	3 and 4	0.08	1	5.94	5.87	90.5%		
SRA	1 and 2	0.04	1	0.194	0.253	92.5%	0.210	^a Equal slopes
	3 and 4	0.08	1	0.270	0.276	90.3%		
^c AQUATRIT	1 and 2	0.04	1	0.062	0.080	92.6%	0.0033 (uncorr.)	^b Unequal slopes
	3 and 4 (uncorrected)	0.08	1	0.234	0.239	89.5%	0.0013 (corr.)	^b Unequal slopes
	3 and 4 (corrected)	0.08	1	0.411	0.987	89.3%		
EDF	1 and 2	0.08	1	1.38	1.63	91.0%	0.932	^a Equal slopes
	3 and 4	0.08	1	1.35	1.59	91.0%		
BIOCHEM	1 and 2	0.08	1	0.090	0.626	84.6%	0.876	^a Equal slopes
	3 and 4	0.08	1	0.095	0.630	85.2%		

^a Equal rates of OBT increase for mussels receiving exposure from sediments and water compared to mussels exposed to water only.

^b OBT uptake rates are predicted to be marginally significantly faster for mussels exposed to OBT via water only compared to those exposed via both water and sediments.

^c Uncorrected values predicted by the AQUATRIT model do not account for elevated tritium concentrations in mussel dietary items in Perch Lake, whereas corrected values assume that mussels are feeding on food with tritium levels that are at steady state with Perch Lake HTO levels.

APPENDIX A

Mussel Uptake Scenario Description, Revision 1

July 2005

A.1. BACKGROUND INFORMATION

Tritium can represent a key radionuclide in the aquatic environment, potentially contributing significantly to the doses received by aquatic non-human biota in surface waters receiving tritium inputs. Although in many cases, steady-state models provide practical tools for estimating free-water tritium concentrations (and to a lesser extent, OBT concentrations), aquatic organisms are occasionally exposed to short-term, elevated tritium concentrations in water when tritium is released accidentally to aquatic systems. Depending upon the nature and the duration of such events, in some cases, steady-state models may or may not reliably predict true organism concentrations.

In general, the rates of free-water tritium uptake and OBT formation are not well known under dynamic exposure conditions, but can be studied by transplanting biomonitoring species, such as freshwater mussels, from areas with background tritium concentrations to those with measurable tritium levels. In this way, changes in tissue free-water tritium (HTO) and OBT concentrations can be monitored to quantify their responses to dynamic exposure conditions.

A.1.1 Study Objective

The objective of this study was to quantify the rates of HTO uptake and OBT formation in freshwater mussels (*Elliptio complanata*) receiving abrupt increases in their tritium exposure levels through transplantation from areas with background tritium concentrations to Perch Lake, a small, Canadian Shield lake receiving chronic, low-level tritium inputs. This information forms the basis for a model-data validation scenario for tritium uptake under dynamic exposure conditions. It complements a previous EMRAS scenario that was designed to test steady-state aquatic tritium models, also based on data from Perch Lake.

A.2. SITE DESCRIPTION

Located on the site of Chalk River Laboratories (CRL), Perch Lake contains trace amounts of tritium (Figure A.1 and Figure A.2). The lake receives tritium inputs via groundwater that is migrating through an extensive sand aquifer from a waste management area (WMA) located approximately 750 m to the north of the lake. The WMA was in operation for approximately 40 years until it was shut down in 1999. The tritium forms a well-defined underground plume that is narrow near the source, but

broadens to a width of approximately 1,000 m by the time it reaches the lake. Tritium, in the form of HTO, discharges into the lake through the sediments from below and also through the Inlet 2 inflowing stream (Figure A.2), which flows above the underground plume. Inlet 1 also shows slightly elevated levels of tritium; however, inflowing streams at Inlets 3, 4 and 5 are all uncontaminated. The rate and distribution of HTO releases to the lake are not known quantitatively, although it is believed that the lake is well-mixed in the vicinity of the mussel transplantation cages, which were deployed near the outflowing stream in the lake.

In terms of its physical size, Perch Lake (Figure A.1) is a small, shallow freshwater Canadian Shield lake, with a maximum fetch of approximately 800 m, a surface area of $4.5 \times 10^5 \text{ m}^2$ and a volume of $9.1 \times 10^5 \text{ m}^3$. The mean depth of the lake is 2.0 m and the maximum depth is 4.1 m. The lake drains a watershed of area $5.65 \times 10^6 \text{ m}^2$ and the residence time of water in the lake is approximately 0.5 years. Perch Lake can be considered unstratified, although there is weak stratification in deeper areas in the summer, when surface waters are approximately 5°C higher than those at lake bottom. The lake is typically ice-covered from early December to mid-April. Based on historical measurements, mean monthly water temperatures are 13, 19, 24, 23, 19 and 11°C for the months of May through October, respectively. Surface water temperatures measured in the vicinity of the mussel transplantation cages in Perch Lake over the course of this study are provided in Table A.1 and Figure A.3. These values are similar to air temperatures measured over the same time periods.

Sediments in the lake are composed of sand and gyttja (decomposing organic material). The mean dry bulk density is approximately 185 kg m^{-3} for Perch Lake sediments, but values vary substantially across the lake depending on the local composition of the sediments. The sediments in the vicinity of the mussel transplantation cages are primarily sandy in nature, with some accumulation of organic matter. These sediments consist of approximately 50% water by weight and the sedimentation rate is $0.16 \text{ kg m}^{-2} \text{ a}^{-1}$ or 0.06 cm a^{-1} .

A.3. STUDY DESIGN

Two pairs of mussel transplantation cages were built and deployed in Perch Lake in early July 2004. These cages contained freshwater mussels originating from a site with background tritium concentrations (as described in Section A.3.3.1) to quantify rates of temporal changes in HTO and OBT in mussel soft tissues. In doing so, two sets of exposure conditions were established, as summarized in Table A.2. These included exposure to tritium via the surface water pathway only (Cages 1 and 2), and exposure via both surface sediments and surface water (Cages 3 and 4). A more detailed description of each cage set-up is provided in Section A.3.3.2 and A3.3.3 below.

A.3.1 Cage Design

Each mussel transplantation cage was constructed with an 8 x 8 design, resulting in a total of 64 compartments per cage (Figure A.4). Each compartment was assigned a unique alphanumeric code (as shown in Table A.3) and one animal was placed into each compartment to facilitate tracking of each animal. Cages were constructed with 2 x 2 cedar and chicken wire, with dimensions of 96 cm (length) x 96 cm (width) x 12 cm (height). Individual cage compartments had surface area dimensions of 12 cm x 12 cm.

A.3.2 Selection of Animals

Freshwater mussels (*Elliptio complanata*) with total shell lengths in the range from 90 to 111 mm were selected for the study during sampling at the reference site. A list of whole animal fresh weights (in g), and total shell lengths, widths and heights (in mm) are provided for each animal in Table A.4 by cage number and compartment for tracking purposes.

A.3.3 Mussel Transplantation

A.3.3.1 Reference Site

Mussels were collected from a reference area with background tritium concentrations at the mouth of the Schyan River (Quebec) in the Ottawa River, upstream of AECL's Chalk River Laboratories site (Figure A.1). Mussels were collected and placed into lidded, plastic buckets containing water from the reference site to prevent uptake of tritium by the mussels prior to initiation of the study. Mussels were then transported to the laboratory on the Chalk River site. Individuals were quickly measured, weighed and alpha-numerically numbered (as shown in Table A.4), and were separated by placing them into labeled nylon bags. Animals were then replaced into the lidded buckets of water from the reference site until initiation of the transplantation, which was carried out on the same day as mussel collection. Concentrations of HTO and OBT measured in surface waters and mussels collected from this background location are provided in Table A.5.

A.3.3.2 Deployment of Mussel Cages 1 and 2 (Water Exposure Pathway)

Mussel Cages 1 and 2 were deployed on 5 July 2005 at 14:00 hours. Cages 1 and 2 were positioned in Perch Lake at a water depth of approximately 0.75 m. These cages were placed on cinder blocks, such that mussels only received tritium exposures through interaction with the water column. Upon initiation of the transplantation study (at time 0), mussels were transferred from the lidded buckets containing water from the reference site to buckets containing water from Perch Lake. In this way, all mussels received initial tritium exposure at approximately the same time, despite the 10 to 15 minute time period required for mussel transfer from buckets to the numbered cage compartments. Mussels began filtering less than five minutes after being placed into the cage compartments. No mussel mortality occurred in Cages 1 or 2 over the course of the 88-day transplantation

study. Algal growth, which accumulated on the cages over the course of the study, was not removed, as it did not appear to alter water flow within the cages.

A.3.3.3 Deployment of Mussel Cages 3 and 4 (Water and Sediment Exposure Pathways)

Mussel Cages 3 and 4 were deployed on 7 July 2004 at 14:00 hours. Cages 3 and 4 were positioned in Perch Lake at the sediment-to-water interface at a water depth of approximately 0.5 m, just inshore of Cages 1 and 2 (Figure A.2), such that mussels received tritium exposure through the sediment and water pathways. Each cage compartment was filled with sandy surface sediments originating from the area surrounding the cages to a depth of approximately 5 to 10 cm, a depth that enabled mussels to position themselves in an upright position with their siphons pointed upwards, as they do in natural systems. The sediments were added to the cages several hours prior to transplantation of the mussels to allow settling of any suspended particulates.

As for Cages 1 and 2, upon initiation of mussel transplantation into Cages 3 and 4 (at time 0), mussels were transferred from the lidded buckets containing water from the reference site to buckets containing water from Perch Lake. Mussels were then placed into the cage compartments and were visually monitored. In general, mussels began positioning themselves in an upright position within five minutes of transplantation. Again, no mussel mortality occurred in Cages 3 or 4 over the course of the 88-day transplantation study.

A.4. STUDY MEASUREMENTS

A.4.1 Tritium Monitoring

A.4.1.1 Collection of Mussel Samples

The composite samples taken at each time point are specified in Table A.6. Mussel samples were collected on an exponential time-step over the course of an 88-day period (as specified in Table A.7). Upon collection, mussels were immediately placed into airtight Mason jars to avoid tritium exchange with the atmosphere. The jars and mussels were later frozen until processing for tritium analysis could be carried out. In general, it was necessary to composite soft tissues from 3 to 4 individuals to gain the biomass required for HTO and OBT analysis. The water content of mussel tissue was 89.0% (by weight), with little variability among individual animals.

A.4.1.2 Collection of Surface Water Samples

Water samples were collected in triplicate at each sampling time in the vicinity of each of the mussel cages (Figure A.2). In doing so, sampling bottles were opened at the depth where the mussels were filtering. The samples were then left standing to allow suspended sediments to settle out and 10 mL of water were subsequently transferred to scintillation vials. HTO concentrations in all water samples were determined by liquid scintillation counting (LSC).

A.4.1.3 Collection of Surface Sediment Samples

Sediment samples were collected by hand at a depth of 5 to 10 cm in the vicinity of the mussel cages at each mussel sampling time. The samples were placed in Ziplock bags that were sealed at depth. Water was extracted from a subset of sediment samples (Table A.7) by freeze-drying and these sediments were analyzed for HTO concentration by LSC. The pressure during freeze-drying fell between 10^{-4} and 10^{-5} Torr and the temperature ranged from 0 to -4°C . The remaining solid material was washed with tritium-free water to remove the exchangeable OBT. Sediments were oven-dried until no change in mass occurred and the dried material was combusted in a combustion tube. The combustion water was analyzed by LSC to quantify OBT concentrations.

A.4.1.4 Collection of Plankton

Plankton samples were collected in the Perch Lake water column on 20 September 2004 just offshore of the cages to quantify tritium levels in mussel dietary items (as an input parameter for modeling purposes). HTO levels of 4153, 4101 and 4068 Bq/L were found in the plankton samples. Corresponding HTO concentrations in Perch Lake surface waters at the time of plankton sampling were 4091, 4066 and 4038 Bq/L. In comparison, an OBT concentration of 2914 ± 42 Bq/L was measured in the composite plankton sample. Note that it was not possible to measure OBT in individual samples due to the relatively large biomass required for OBT analysis.

A.4.2 Monitoring of Fluctuations in Water Temperature

Perch Lake surface water temperatures were taken continuously using a temperature probe set to integrate values over 5-minute time intervals. The probe was positioned a few centimetres above the sediment-water interface.

A.5. INPUT DATA

Measured HTO concentrations in water and mussel soft tissues collected at the background location are provided in Table A.5. In addition, water and sediment tritium levels measured at each sampling time are summarized in Table A.7. Plankton HTO and OBT data are listed in Section A.4.1.4 above.

In cases where more than one value is listed for a given parameter, separate composite samples were taken close to the same location to facilitate measurement of variability.

A.5.1 Uncertainties

Counting errors in the HTO concentrations in Perch Lake surface waters and sediments were generally less than 2%. Counting errors for OBT concentrations are typically less than 5%, although additional uncertainty can arise due to difficulties in removing exchangeable OBT from the samples and during the combustion process. The total uncertainty in the OBT measurements is estimated to be approximately 25%. Differences among replicate samples from the same location may be larger because of natural variability.

A.6. SCENARIO CALCULATIONS

Using the information provided in the Sections above, participants in the scenario are asked to calculate:

- (i.) HTO and non-exchangeable OBT concentrations (Bq/L) in mussels exposed only via water (i.e. in Cages 1 and 2) for each measurement time-point, as specified in Table A.8;
- (ii.) HTO and non-exchangeable OBT concentrations (Bq/L) in mussels exposed via both water and sediments (i.e. in Cages 3 and 4) for each measurement time-point, as specified in Table A.9; and
- (iii.) 95% confidence intervals on all predictions in (i) - (ii).

Results should be submitted using Table A.8 and Table A.9.

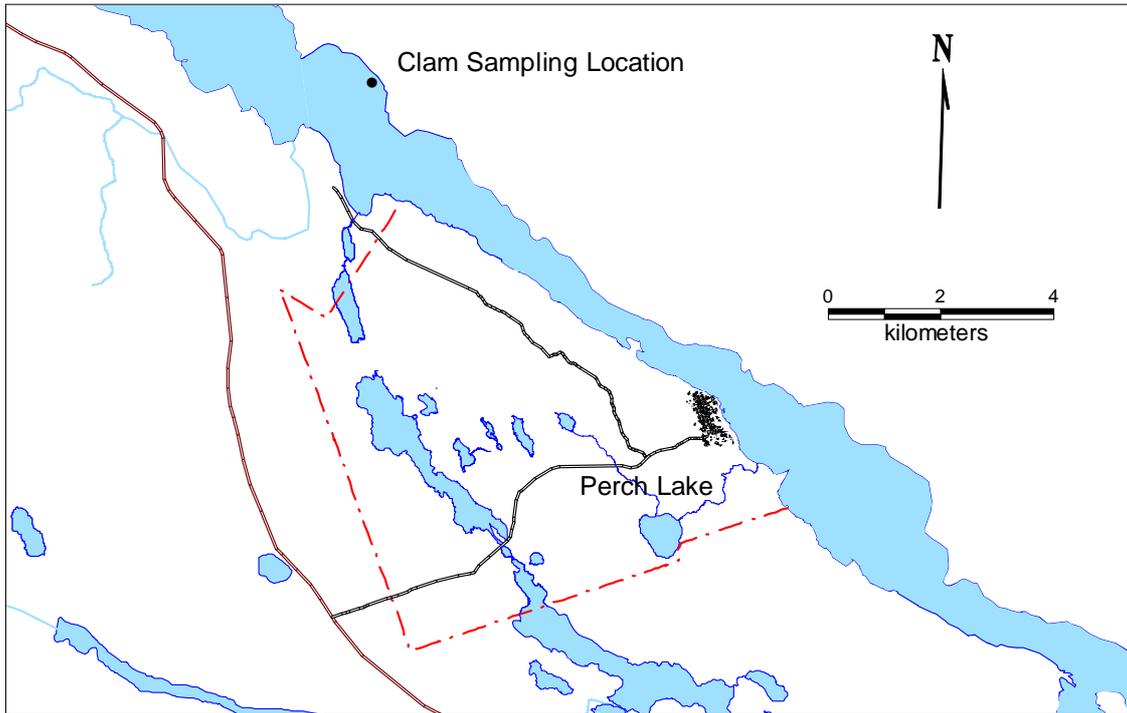


Figure A.1: Map depicting the location of the reference site in the Ottawa River where freshwater mussels (*Elliptio complanata*) were collected, relative to the site of mussel transplantation in Perch Lake on AECL's Chalk River Laboratories site.

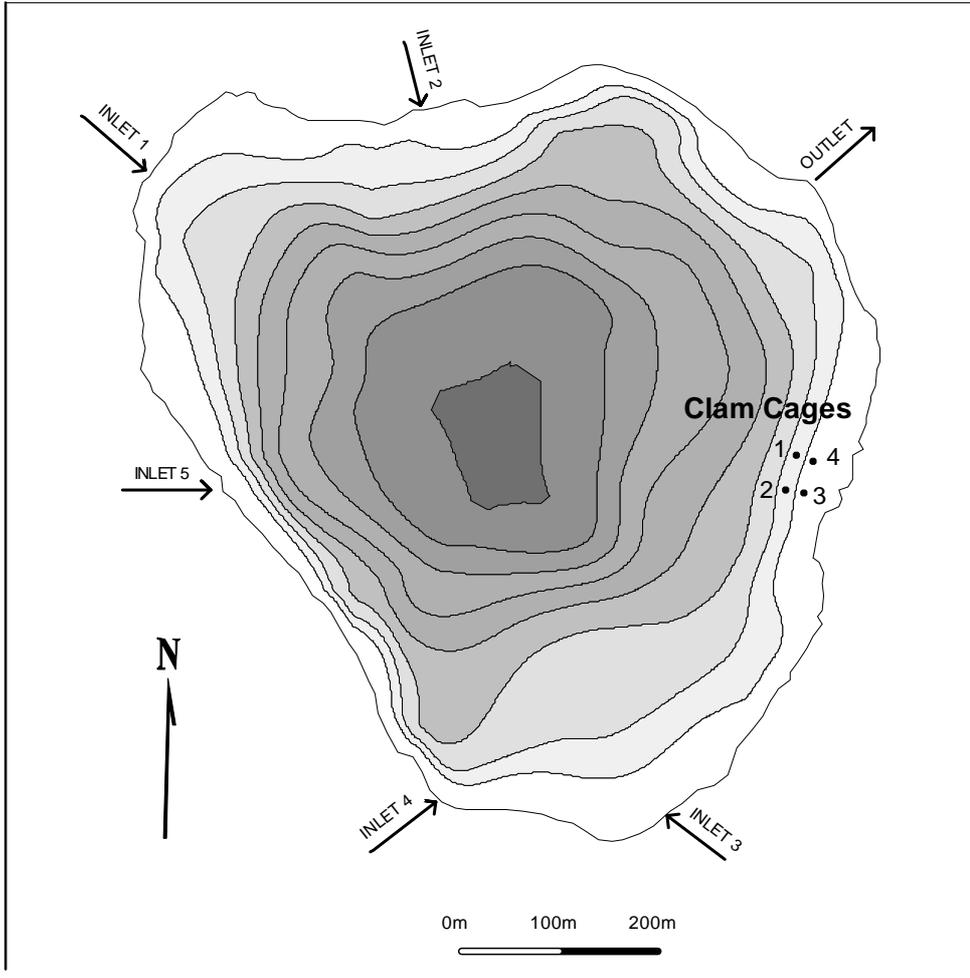


Figure A.2: Map of Perch Lake depicting the location of inflowing and outflowing streams, depth contours (in metres) and locations of mussel transplantation cages.

Perch Lake Water Temperature Summer 2004

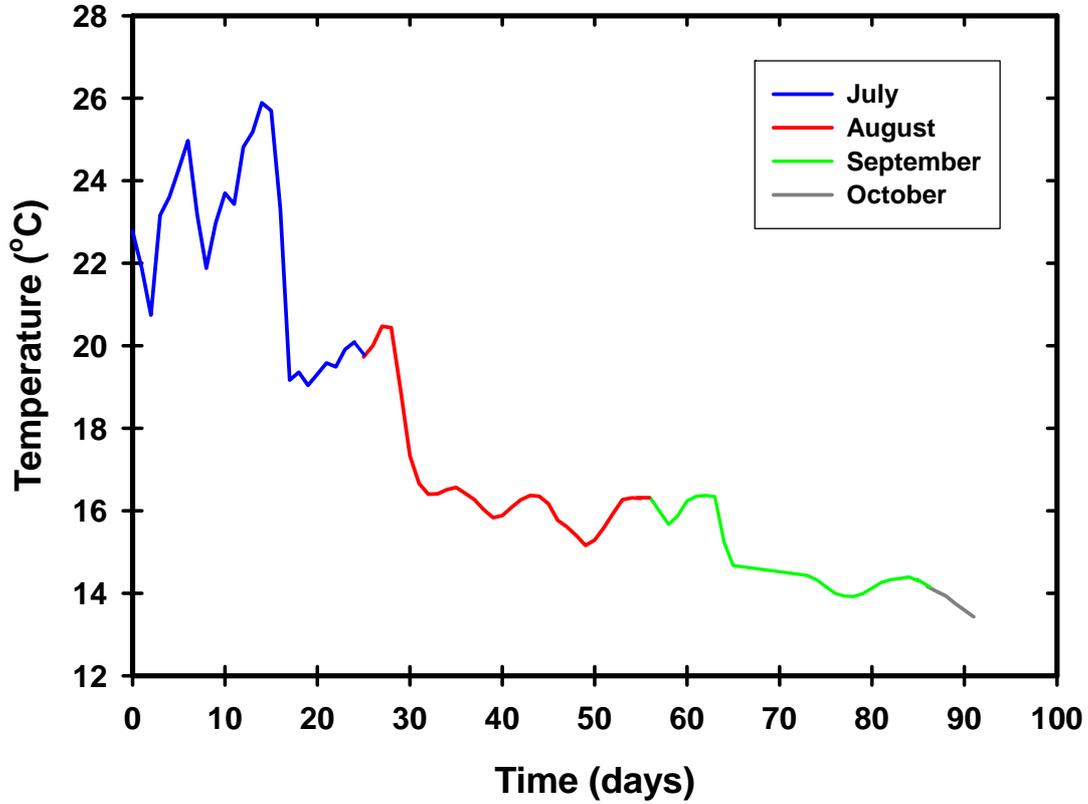


Figure A.3: Depiction of changes in Perch Lake water temperatures over the course of the mussel transplantation study. Temperature measurements were integrated over 5-minute time intervals between 5 July 2004 and 6 October 2004. The experiment starting time for Cages 1 and 2 was 5 July 2004 at 14:00, whereas the starting time for Cages 3 and 4 was 7 July 2004 at 14:00. Comparable trends were observed for air temperatures.

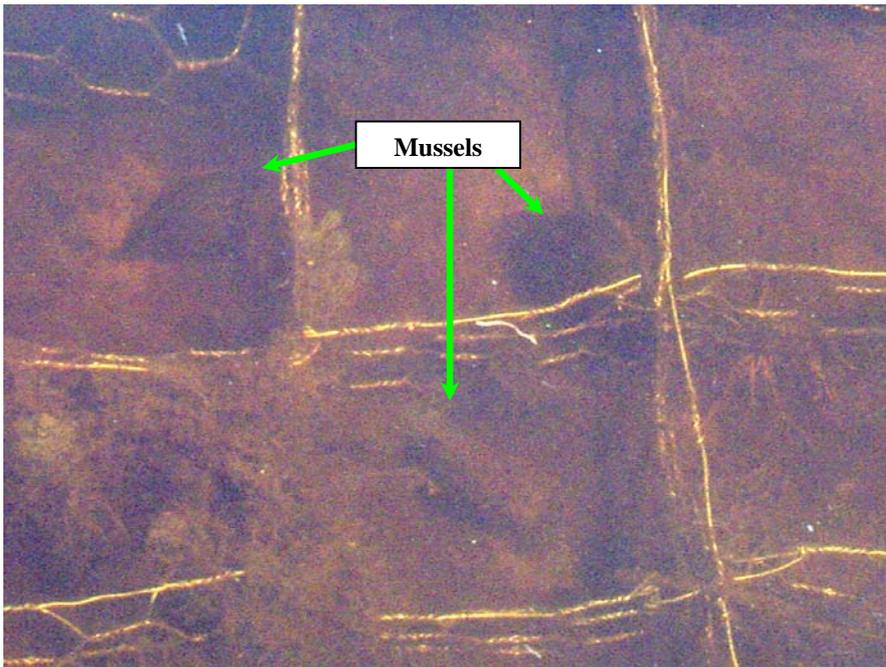


Figure A.4: Photographs depicting the design of the mussel transplantation cages with sediment and water tritium exposure (top panel) and exposure from water only (bottom panel).

Table A.1: Compilation of daily statistical Perch Lake water temperature (T) data, based on integrated measurements taken over 5-minute intervals. Temperature data were not available for the period between September 11 and 17 due to a problem with the temperature probe. Raw temperature data are available upon request.

Date	Mean Temperature T (°C)	n	Standard Error of 5-Minute Values (°C)
07-Jul-04	22.77	150	0.127
08-Jul-04	21.88	288	0.0950
09-Jul-04	20.74	288	0.0658
10-Jul-04	23.16	288	0.192
11-Jul-04	23.61	288	0.0900
12-Jul-04	24.28	288	0.102
13-Jul-04	24.97	288	0.0903
14-Jul-04	23.19	288	0.0549
15-Jul-04	21.88	288	0.0523
16-Jul-04	22.97	288	0.110
17-Jul-04	23.70	288	0.108
18-Jul-04	23.43	288	0.120
19-Jul-04	24.82	288	0.100
20-Jul-04	25.18	288	0.160
21-Jul-04	25.89	288	0.0916
22-Jul-04	25.70	288	0.0802
23-Jul-04	23.31	288	0.127
24-Jul-04	19.17	288	0.0574
25-Jul-04	19.36	288	0.0088
26-Jul-04	19.04	288	0.0206
27-Jul-04	19.31	288	0.0064
28-Jul-04	19.58	288	0.0058
29-Jul-04	19.49	288	0.0088
30-Jul-04	19.91	288	0.0041
31-Jul-04	20.09	288	0.0029
01-Aug-04	19.73	288	0.0122
02-Aug-04	20.00	288	0.0056
03-Aug-04	20.47	288	0.0065
04-Aug-04	20.44	288	0.0197
05-Aug-04	18.93	288	0.0635
06-Aug-04	17.33	288	0.0172
07-Aug-04	16.66	288	0.0124
08-Aug-04	16.40	288	0.0013
09-Aug-04	16.41	288	0.0010
10-Aug-04	16.51	288	0.0028
11-Aug-04	16.57	288	0.0015
12-Aug-04	16.42	288	0.0032
13-Aug-04	16.27	288	0.0025
14-Aug-04	16.03	288	0.0072
15-Aug-04	15.83	288	0.0005

Date	Mean Temperature T (°C)	n	Standard Error of 5-Minute Values (°C)
16-Aug-04	15.88	288	0.0022
17-Aug-04	16.08	288	0.0041
18-Aug-04	16.26	288	0.0029
19-Aug-04	16.37	288	0.0012
20-Aug-04	16.35	288	0.0061
21-Aug-04	16.17	288	0.0045
22-Aug-04	15.77	288	0.0129
23-Aug-04	15.61	288	0.0040
24-Aug-04	15.40	288	0.0167
25-Aug-04	15.16	288	0.0014
26-Aug-04	15.29	288	0.0047
27-Aug-04	15.59	288	0.0051
28-Aug-04	15.93	288	0.0063
29-Aug-04	16.27	288	0.0040
30-Aug-04	16.31	288	0.0003
31-Aug-04	16.30	288	0.0004
01-Sep-04	16.32	288	0.0011
02-Sep-04	15.99	288	0.0172
03-Sep-04	15.67	288	0.0010
04-Sep-04	15.88	288	0.0072
05-Sep-04	16.24	288	0.0037
06-Sep-04	16.35	288	0.0014
07-Sep-04	16.37	288	0.0017
08-Sep-04	16.34	288	0.0016
09-Sep-04	15.24	288	0.0390
10-Sep-04	14.67	177	0.0022
18-Sep-04	14.43	156	0.0007
19-Sep-04	14.33	288	0.0029
20-Sep-04	14.16	288	0.0033
21-Sep-04	14.00	288	0.0023
22-Sep-04	13.93	288	0.0010
23-Sep-04	13.92	288	0.0006
24-Sep-04	13.99	288	0.0017
25-Sep-04	14.12	288	0.0023
26-Sep-04	14.26	288	0.0021
27-Sep-04	14.33	288	0.0005
28-Sep-04	14.36	288	0.0006
29-Sep-04	14.39	288	0.0004
30-Sep-04	14.30	288	0.0026
01-Oct-04	14.16	288	0.0024
02-Oct-04	14.04	288	0.0021
03-Oct-04	13.93	288	0.0023
04-Oct-04	13.75	288	0.0032
05-Oct-04	13.59	288	0.0028
06-Oct-04	13.43	171	0.0034

Table A.2: Transplanted freshwater mussel (*Elliptio complanata*) exposure pathways under the various test conditions.

Cage No.	Exposure Medium	
	Water	Sediments
1	X	-
2	X	-
3	X	X
4	X	X

Table A.3: Layout of mussel transplantation cages and mussel numbering scheme. Cages were set up as a matrix and individual mussels were numbered as alphanumerical coordinates of alphabetical ‘columns’ and numerical ‘row’ numbers to facilitate tracking of each mussel in terms of tritium uptake rates relative to mussel body size.

Column Row	A	B	C	D	E	F	G	H
1	A1	B1	C1	D1	E1	F1	G1	H1
2	A2	B2	C2	D2	E2	F2	G2	H2
3	A3	B3	C3	D3	E3	F3	G3	H3
4	A4	B4	C4	D4	E4	F4	G4	H4
5	A5	B5	C5	D5	E5	F5	G5	H5
6	A6	B6	C6	D6	E6	F6	G6	H6
7	A7	B7	C7	D7	E7	F7	G7	H7
8	A8	B8	C8	D8	E8	F8	G8	H8

Table A.4: Free-water tritium (HTO) and organically-bound tritium (OBT) concentrations in various sample types collected at the background location in the Ottawa River, upstream of AECL’s Chalk River Laboratories site. Values measured for mussels represent the initial tritium levels at the start of the study.

Sample Type	HTO (Bq/L)	OBT (Bq/L)
Surface Water	< 10	Not applicable
Freshwater Mussels	< 10	< 15

Table A.5: Weight and length measurements of mussel specimens transplanted from the Ottawa River upstream of CRL to Perch Lake.

Cell No.	Cage No.	<i>Mussel Measurements</i>			
		Fresh Weight (g)	Shell Length (mm)	Shell Width (mm)	Shell Height (mm)
A1	Cage No. 1	64.40	96	46	24
	Cage No. 2	60.03	92	49	23
	Cage No. 3	100.77	111	58	28
	Cage No. 4	78.33	98	49	24
A2	Cage No. 1	95.19	98	54	28
	Cage No. 2	57.35	92	45	21
	Cage No. 3	74.09	96	51	27
	Cage No. 4	64.90	95	49	25
A3	Cage No. 1	62.94	90	48	25
	Cage No. 2	68.62	93	46	26
	Cage No. 3	122.57	109	57	33
	Cage No. 4	97.13	103	53	27
A4	Cage No. 1	83.50	103	49	27
	Cage No. 2	61.38	90	45	24
	Cage No. 3	62.44	94	46	26
	Cage No. 4	60.93	94	45	24
A5	Cage No. 1	79.23	99	50	26
	Cage No. 2	91.42	105	51	30
	Cage No. 3	85.65	103	50	28
	Cage No. 4	90.77	105	53	28
A6	Cage No. 1	102.05	102	56	27
	Cage No. 2	58.94	93	47	23
	Cage No. 3	87.57	104	56	28
	Cage No. 4	77.47	103	51	25
A7	Cage No. 1	69.89	95	49	24

Cell No.	Cage No.	<i>Mussel Measurements</i>			
		Fresh Weight (g)	Shell Length (mm)	Shell Width (mm)	Shell Height (mm)
	Cage No. 2	74.51	96	52	26
	Cage No. 3	56.50	92	52	20
	Cage No. 4	100.44	109	57	29
A8	Cage No. 1	83.58	96	51	27
	Cage No. 2	72.89	94	50	26
	Cage No. 3	61.72	92	46	25
	Cage No. 4	70.48	90	51	25
B1	Cage No. 1	73.07	96	46	27
	Cage No. 2	90.96	100	54	30
	Cage No. 3	82.79	101	53	26
	Cage No. 4	69.16	90	49	25
B2	Cage No. 1	75.31	95	48	26
	Cage No. 2	98.10	105	54	32
	Cage No. 3	86.19	107	55	25
	Cage No. 4	117.87	109	59	31
B3	Cage No. 1	77.75	95	51	27
	Cage No. 2	79.26	95	52	29
	Cage No. 3	75.66	99	53	27
	Cage No. 4	73.90	100	51	26
B4	Cage No. 1	94.55	104	54	28
	Cage No. 2	73.14	94	51	27
	Cage No. 3	72.95	98	51	26
	Cage No. 4	85.76	102	52	26
B5	Cage No. 1	66.31	94	49	26
	Cage No. 2	70.63	94	53	27
	Cage No. 3	74.28	103	51	27
	Cage No. 4	73.64	100	49	24
B6	Cage No. 1	98.34	106	56	27

Cell No.	Cage No.	<i>Mussel Measurements</i>			
		Fresh Weight (g)	Shell Length (mm)	Shell Width (mm)	Shell Height (mm)
	Cage No. 2	62.84	90	51	35
	Cage No. 3	101.33	110	54	30
	Cage No. 4	83.43	104	52	25
B7	Cage No. 1	70.41	95	49	26
	Cage No. 2	65.22	96	47	27
	Cage No. 3	91.92	100	54	28
	Cage No. 4	77.91	93	50	26
B8	Cage No. 1	70.29	103	47	22
	Cage No. 2	70.75	90	51	29
	Cage No. 3	74.20	99	50	28
	Cage No. 4	78.51	98	49	26
C1	Cage No. 1	67.95	97	47	25
	Cage No. 2	73.15	100	46	26
	Cage No. 3	102.75	108	53	31
	Cage No. 4	69.39	95	47	27
C2	Cage No. 1	80.67	104	54	25
	Cage No. 2	62.98	94	58	26
	Cage No. 3	68.65	97	47	24
	Cage No. 4	84.76	100	50	27
C3	Cage No. 1	57.44	93	45	23
	Cage No. 2	77.36	100	55	26
	Cage No. 3	71.25	99	48	27
	Cage No. 4	57.55	95	47	21
C4	Cage No. 1	79.36	104	52	25
	Cage No. 2	79.90	98	48	28
	Cage No. 3	83.91	105	53	29
	Cage No. 4	94.57	105	55	26
C5	Cage No. 1	73.39	96	50	25

Cell No.	Cage No.	<i>Mussel Measurements</i>			
		Fresh Weight (g)	Shell Length (mm)	Shell Width (mm)	Shell Height (mm)
	Cage No. 2	63.48	95	52	23
	Cage No. 3	84.51	103	51	29
	Cage No. 4	67.19	102	50	22
C6	Cage No. 1	86.02	99	49	30
	Cage No. 2	81.52	100	52	26
	Cage No. 3	78.38	104	51	26
	Cage No. 4	94.18	105	50	29
C7	Cage No. 1	83.06	101	52	26
	Cage No. 2	82.38	102	59	30
	Cage No. 3	70.38	98	47	27
	Cage No. 4	78.38	100	51	27
C8	Cage No. 1	74.35	101	46	26
	Cage No. 2	119.84	109	57	33
	Cage No. 3	81.21	104	54	27
	Cage No. 4	80.26	98	50	27
D1	Cage No. 1	101.37	103	58	27
	Cage No. 2	113.44	110	56	30
	Cage No. 3	117.32	106	60	30
	Cage No. 4	70.64	95	50	24
D2	Cage No. 1	101.61	101	55	29
	Cage No. 2	96.75	104	56	30
	Cage No. 3	78.61	102	55	28
	Cage No. 4	80.66	99	52	26
D3	Cage No. 1	83.65	102	50	25
	Cage No. 2	97.71	101	59	30
	Cage No. 3	77.04	100	50	26
	Cage No. 4	81.01	101	51	25
D4	Cage No. 1	68.54	96	49	29

Cell No.	Cage No.	<i>Mussel Measurements</i>			
		Fresh Weight (g)	Shell Length (mm)	Shell Width (mm)	Shell Height (mm)
	Cage No. 2	116.83	110	51	33
	Cage No. 3	71.61	94	50	26
	Cage No. 4	82.94	104	51	26
D5	Cage No. 1	69.29	95	49	26
	Cage No. 2	68.78	93	53	25
	Cage No. 3	103.58	109	55	30
	Cage No. 4	78.11	99	51	25
D6	Cage No. 1	78.06	99	49	27
	Cage No. 2	98.91	104	50	30
	Cage No. 3	74.73	93	53	24
	Cage No. 4	86.86	105	51	26
D7	Cage No. 1	74.73	99	50	25
	Cage No. 2	56.23	94	50	24
	Cage No. 3	91.28	99	54	29
	Cage No. 4	74.43	100	51	26
D8	Cage No. 1	68.01	95	45	25
	Cage No. 2	78.77	94	52	28
	Cage No. 3	76.94	96	51	24
	Cage No. 4	67.74	91	45	26
E1	Cage No. 1	70.48	101	50	23
	Cage No. 2	94.40	100	58	30
	Cage No. 3	75.84	100	51	27
	Cage No. 4	56.26	93	46	24
E2	Cage No. 1	83.36	104	53	26
	Cage No. 2	93.48	100	52	30
	Cage No. 3	85.21	96	51	29
	Cage No. 4	74.88	94	52	25
E3	Cage No. 1	75.97	96	50	27

Cell No.	Cage No.	<i>Mussel Measurements</i>			
		Fresh Weight (g)	Shell Length (mm)	Shell Width (mm)	Shell Height (mm)
	Cage No. 2	87.74	104	53	29
	Cage No. 3	108.61	101	54	34
	Cage No. 4	67.46	100	50	21
E4	Cage No. 1	94.02	106	55	32
	Cage No. 2	84.80	101	54	29
	Cage No. 3	121.49	106	58	32
	Cage No. 4	82.10	91	50	28
E5	Cage No. 1	68.08	97	48	25
	Cage No. 2	78.27	98	50	29
	Cage No. 3	71.57	98	50	25
	Cage No. 4	93.52	106	54	26
E6	Cage No. 1	94.80	99	50	29
	Cage No. 2	59.17	90	48	24
	Cage No. 3	67.72	94	49	26
	Cage No. 4	79.62	100	54	24
E7	Cage No. 1	76.23	96	54	25
	Cage No. 2	90.52	102	57	29
	Cage No. 3	67.71	98	46	25
	Cage No. 4	68.97	94	47	26
E8	Cage No. 1	72.53	96	48	26
	Cage No. 2	84.61	102	53	28
	Cage No. 3	91.71	100	54	28
	Cage No. 4	64.47	94	48	25
F1	Cage No. 1	82.47	100	56	25
	Cage No. 2	106.65	108	55	31
	Cage No. 3	118.56	106	56	35
	Cage No. 4	72.55	102	50	23
F2	Cage No. 1	71.93	92	45	26

Cell No.	Cage No.	<i>Mussel Measurements</i>			
		Fresh Weight (g)	Shell Length (mm)	Shell Width (mm)	Shell Height (mm)
	Cage No. 2	83.38	100	53	30
	Cage No. 3	93.37	108	55	27
	Cage No. 4	75.37	97	51	24
F3	Cage No. 1	64.14	95	46	25
	Cage No. 2	70.93	99	49	26
	Cage No. 3	84.16	98	54	28
	Cage No. 4	77.31	100	50	27
F4	Cage No. 1	64.66	90	43	27
	Cage No. 2	62.23	94	52	25
	Cage No. 3	52.74	95	44	22
	Cage No. 4	56.74	92	46	24
F5	Cage No. 1	57.42	96	46	20
	Cage No. 2	66.86	94	52	27
	Cage No. 3	86.67	96	56	27
	Cage No. 4	61.29	93	48	23
F6	Cage No. 1	62.56	91	45	24
	Cage No. 2	81.23	96	55	28
	Cage No. 3	87.95	99	51	27
	Cage No. 4	55.34	101	50	26
F7	Cage No. 1	77.95	96	50	25
	Cage No. 2	86.17	100	50	30
	Cage No. 3	78.95	101	50	25
	Cage No. 4	88.66	105	51	25
F8	Cage No. 1	103.22	102	52	32
	Cage No. 2	80.08	98	50	27
	Cage No. 3	78.25	96	56	27
	Cage No. 4	79.17	96	50	26
G1	Cage No. 1	93.02	100	50	29

Cell No.	Cage No.	<i>Mussel Measurements</i>			
		Fresh Weight (g)	Shell Length (mm)	Shell Width (mm)	Shell Height (mm)
	Cage No. 2	84.70	102	56	28
	Cage No. 3	75.21	92	49	29
	Cage No. 4	97.28	101	53	28
G2	Cage No. 1	87.85	100	51	27
	Cage No. 2	81.72	96	52	29
	Cage No. 3	88.85	100	50	29
	Cage No. 4	68.91	100	49	24
G3	Cage No. 1	81.58	98	52	27
	Cage No. 2	92.11	101	59	28
	Cage No. 3	73.52	95	48	27
	Cage No. 4	57.64	95	43	25
G4	Cage No. 1	78.90	103	49	25
	Cage No. 2	76.98	101	49	28
	Cage No. 3	96.64	104	51	30
	Cage No. 4	65.54	95	49	25
G5	Cage No. 1	81.23	98	50	26
	Cage No. 2	85.68	103	54	27
	Cage No. 3	87.76	99	52	26
	Cage No. 4	59.86	93	46	24
G6	Cage No. 1	75.92	104	50	26
	Cage No. 2	69.04	93	49	24
	Cage No. 3	87.04	94	51	26
	Cage No. 4	78.69	101	48	26
G7	Cage No. 1	82.61	99	51	26
	Cage No. 2	102.42	109	58	28
	Cage No. 3	90.70	105	52	29
	Cage No. 4	77.30	95	50	28
G8	Cage No. 1	101.38	101	55	30

Cell No.	Cage No.	<i>Mussel Measurements</i>			
		Fresh Weight (g)	Shell Length (mm)	Shell Width (mm)	Shell Height (mm)
	Cage No. 2	111.92	105	54	32
	Cage No. 3	77.33	93	51	27
	Cage No. 4	71.08	95	49	26
H1	Cage No. 1	99.11	99	51	29
	Cage No. 2	58.79	95	49	23
	Cage No. 3	78.30	96	50	27
	Cage No. 4	88.84	99	52	28
H2	Cage No. 1	102.84	106	58	29
	Cage No. 2	76.84	100	52	27
	Cage No. 3	73.16	101	51	22
	Cage No. 4	70.65	97	48	25
H3	Cage No. 1	89.06	105	54	27
	Cage No. 2	91.36	105	57	27
	Cage No. 3	76.54	97	50	27
	Cage No. 4	62.94	91	48	25
H4	Cage No. 1	71.87	92	48	24
	Cage No. 2	97.37	104	60	30
	Cage No. 3	78.72	94	49	27
	Cage No. 4	78.80	100	50	26
H5	Cage No. 1	99.63	107	59	29
	Cage No. 2	82.38	102	54	29
	Cage No. 3	93.95	105	54	28
	Cage No. 4	59.08	91	46	23
H6	Cage No. 1	86.78	101	50	27
	Cage No. 2	79.57	96	51	30
	Cage No. 3	79.56	101	51	25
	Cage No. 4	75.75	98	51	25
H7	Cage No. 1	87.75	100	51	28

Cell No.	Cage No.	<i>Mussel Measurements</i>			
		Fresh Weight (g)	Shell Length (mm)	Shell Width (mm)	Shell Height (mm)
	Cage No. 2	92.28	99	55	30
	Cage No. 3	87.52	102	51	26
	Cage No. 4	76.51	94	50	25
H8	Cage No. 1	99.67	107	56	27
	Cage No. 2	67.62	96	49	26
	Cage No. 3	73.50	101	48	25
	Cage No. 4	65.86	93	49	25

Table A.6: Individual mussels collected from Cages 1 to 4 in Perch Lake at each sampling time.

Time After Mussel Transplantation	Water Only Exposure		Sediment and Water Exposure		Comments
	<i>Cage 1</i>	<i>Cage 2</i>	<i>Cage 3</i>	<i>Cage 4</i>	
0 hours (all cages)	n.a.	n.a.	n.a.	n.a.	Duplicate samples taken for QA purposes.
	n.a.	n.a.	n.a.	n.a.	
	n.a.	n.a.	n.a.	n.a.	
1 hour (all cages)	A6	G5	C6	B3	
	G2	H3	E5	D8	
	H5	H7	H8	F5	
2 hours (all cages)	C4	B4	G2	A6	
	A8	E7	B5	E1	
	F3	H2	E8	E4	
4 hours (all cages)	A7	A3	A5	C3	
	B3	C6	E3	E2	
	D7	E3	G7	E6	
7 hours (all cages)	B5	B6	C5	D2	
	E6	D5	A8	E7	
	G3	E4	H3	G4	
19 hours (all cages)	B4	B3	A1	B5	Duplicate samples taken for QA purposes.
	D2	B8	C3	B6	
	H4	F1	D4	D1	
	C7	A8	E7	E5	
	E5	D1	G4	F2	
24 hours (all cages)	D6	F2	A3	A8	
	E3	F5	B8	C1	
	G5	D6	G5	F4	
48 hours (all cages)	C2	C4	B2	A1	
	D8	E6	E6	D5	
	F5	G3	D5	F6	
96 hours (all cages)	B2	A4	B1	A3	Duplicate samples taken for QA purposes.

Time After Mussel Transplantation	Water Only Exposure		Sediment and Water Exposure		Comments
	<i>Cage 1</i>	<i>Cage 2</i>	<i>Cage 3</i>	<i>Cage 4</i>	
	B8	A6	C4	B7	
	D5	C5	D6	D3	
	E1	E2	E2	G5	
	F4	F8	H4	C6	
	H8	G4	H7	H2	
8 days (all cages)	C8	A7	A2	A4	
	F6	C1	D7	D7	
	G4	H5	F7	F3	
14 days (all cages)	B6	B1	A4	C4	Duplicate samples taken for QA purposes.
	B7	B7	B6	B1	
	C1	D4	E1	C7	
	E7	D8	F6	F7	
	G6	G1	F8	G1	
	H6	G7	G1	H7	
18 days (Cages 1 & 2)	D3	D3	B7	B2	Cages 3 and 4 sampled 19 days after transplantation.
19 days (Cages 3 & 4)	F2	C7	F4	D6	
	G7	E8	H6	H8	
25 days (Cages 1 & 2)	C6	C8	A7	C5	Duplicate samples taken for QA purposes. Cages 3 and 4 sampled 27 days after transplantation.
27 days (Cages 3 & 4)	D4	D2	C1	C8	
	E4	D7	C2	F1	
	F7	E5	D8	G6	
	G1	H4	F3	H1	
	G8	F6	H5	H6	
36 days (Cages 1 & 2)	A5	A2	C8	B8	Cages 3 and 4 sampled 35 days after transplantation.
35 days (Cages 3 & 4)	D1	C3	E4	G7	
	H1	H8	H2	E3	
42 days (Cages 1 & 2)	C3	B2	A6	A2	Duplicate samples taken for QA purposes. Cages 3 and 4 sampled 41 days after transplantation.
41 days (Cages 3 & 4)	C5	B5	B4	C2	
	E2	E1	D1	G2	
	F1	F3	D2	G3	
	H3	G8	G3	H4	
	E8	H6	G8	H5	
86 days (Cages 1 & 2)	A1	A1	B3	A7	

Time After Mussel Transplantation	Water Only Exposure		Sediment and Water Exposure		Comments
	<i>Cage 1</i>	<i>Cage 2</i>	<i>Cage 3</i>	<i>Cage 4</i>	
84 days (Cages 3 & 4)	A2	A5	C7	B4	
	A3	C2	D3	D4	
	A4	F4	F1	E8	
	F8	G2	F2	G8	
	B1	G6	H1	H3	
	H2				

Table A.7: Tritium input data for use in the Perch Lake dynamic mussel transplantation scenario.

Time After Mussel Transplantation	Water HTO (Bq/L)		Surface Sediments (Between Cages 1 and 2)		Water HTO (Bq/L)		Surface Sediments Between Cages 3 and 4	
	Cage 1	Cage 2	HTO (Bq/L)	OBT (Bq/L)	Cage 3	Cage 4	HTO (Bq/L)	OBT (Bq/L)
0 hour (all cages)	4,800	4,787	-	-	4,645	4,799	-	-
	4,847	4,880	-	-	4,688	4,763	-	-
	4,689	4,775	-	-	4,656	4,636	-	-
1 hour (all cages)	4,735	4,829	-	-	4,646	4,729	4,310	1,020 ± 26
	4,785	4,685	-	-	4,689	4,792	4,296	
	4,830	4,734	-	-	4,844	4,795	-	-
2 hours (all cages)	4,637	4,711	3,926	994 ± 23	4,762	4,715	-	-
	4,641	4,625	3,961		4,685	4,638	-	-
	4,575	4,795	-	-	4,766	4,709	-	-
4 hours (all cages)	4,718	4,636	-	-	4,661	4,718	-	-
	4,705	4,747	-	-	4,711	4,835	-	-
	4,598	4,683	-	-	4,758	4,660	-	-
7 hours (all cages)	4,804	4,611	-	-	4,753	4,688	-	-
	4,638	4,745	-	-	4,653	4,769	-	-
	4,752	4,719	-	-	4,566	4,685	-	-
19 hours (all cages)	4,821	4,796	-	-	4,456	4,378	-	-
	4,784	4,840	-	-	4,350	4,356	-	-
	4,743	4,716	-	-	4,329	4,339	-	-

Time After Mussel Transplantation	Water HTO (Bq/L)		Surface Sediments (Between Cages 1 and 2)		Water HTO (Bq/L)		Surface Sediments Between Cages 3 and 4	
	Cage 1	Cage 2	HTO (Bq/L)	OBT (Bq/L)	Cage 3	Cage 4	HTO (Bq/L)	OBT (Bq/L)
24 hours (all cages)	4,683	4,734	4,015	700 ± 7	4,464	4,522	3,802	1,248 ± 50
	4,832	4,677	4,025	-	4,371	4,478	3,854	-
	4,683	4,774	-	-	4,386	4,427	-	-
48 hours (all cages)	4,645	4,799	-	-	4,429	4,503	-	-
	4,688	4,763	-	-	4,371	4,329	-	-
	4,656	4,636	-	-	4,574	4,648	-	-
96 hours (all cages)	4,597	4,615	-	-	4,526	4,549	-	-
	4,650	4,609	-	-	4,547	4,722	-	-
	4,699	4,605	-	-	4,617	4,534	-	-
8 days (all cages)	4,678	4,634	-	-	4,431	4,270	-	-
	4,749	4,697	-	-	4,312	4,348	-	-
	4,696	4,683	-	-	4,200	4,376	-	-
14 days (all cages)	4,410	4,472	3,993	571 ± 9	4,150	4,212	3,845	1,403 ± 66
	4,417	4,533	3,919	-	4,128	4,182	3,795	-
	4,298	4,365	-	-	4,171	4,137	-	-
18 days (Cages 1 & 2)	4,438	4,347	-	-	4,470	4,415	-	-
19 days (Cages 3 & 4)	4,367	4,337	-	-	4,385	4,417	-	-
	4,276	4,347	-	-	4,374	4,443	-	-
25 days (Cages 1 & 2)	4,383	4,329	-	-	4,136	4,073	-	-
27 days (Cages 3 & 4)	4,412	4,420	-	-	3,985	4,088	-	-
	4,299	4,359	-	-	4,132	4,143	-	-

Time After Mussel Transplantation	Water HTO (Bq/L)		Surface Sediments (Between Cages 1 and 2)		Water HTO (Bq/L)		Surface Sediments Between Cages 3 and 4	
	Cage 1	Cage 2	HTO (Bq/L)	OBT (Bq/L)	Cage 3	Cage 4	HTO (Bq/L)	OBT (Bq/L)
36 days (Cages 1 & 2)	4,238	4,393	-	-	4,150	4,328	3,894	1,159 ± 33
35 days (Cages 3 & 4)	4,268	4,313	-	-	4,176	4,272	3,876	-
	4,387	4,191	-	-	4,180	4,281	-	-
42 days (Cages 1 & 2)	4,102	4,173	3,802	704 ± 17	4,069	4,088	-	-
41 days (Cages 3 & 4)	4,182	4,137	3,857	-	4,094	4,066	-	-
	4,109	4,079	-	-	3,977	3,991	-	-
^a 77 days	4,091	-	-	-	-	-	-	-
	4,066	-	-	-	-	-	-	-
	4,038	-	-	-	-	-	-	-
86 days (Cages 1 & 2)	3,930	4,088	-	-	4,046	3,955	3,274	1,829 ± 28 (Cage 3)
84 days (Cages 3 & 4)	3,973	3,949	-	-	4,038	4,062	3,840	1,981 ± 57 (Cage 4)

Measurement error for HTO was <1%.

^a Triplicate water samples were collected in the area where plankton samples were taken. Water data are likely representative of a well-mixed condition in the lake.

Table A.8: Model output parameters for the dynamic Perch Lake mussel transplantation scenario.

Time After Mussel Transplantation	<i>Exposure to Surface Water Only (Cages 1 and 2)</i>			
	HTO Mussel Concentration (Bq/L)	± 95% Confidence Interval	OBT Mussel Concentration (Bq/L)	± 95% Confidence Interval
0 hour	given	given	given	given
1 hour				
2 hours				
4 hours				
7 hours				
19 hours				
24 hours				
48 hours				
96 hours				
8 days				
14 days				
18 days				
25 days				
36 days				
42 days				
77 days				
86 days				

Table A.9: Model output parameters for the dynamic Perch Lake mussel transplantation scenario.

Time After Mussel Transplantation	<i>Exposure to both Surface Water and Sediments (Cages 3 and 4)</i>			
	HTO Mussel Concentration (Bq/L)	± 95% Confidence Interval	OBT Mussel Concentration (Bq/L)	± 95% Confidence Interval
0 hour	given	given	given	given
1 hour				
2 hours				
4 hours				
7 hours				
19 hours				
24 hours				
48 hours				
96 hours				
8 days				
14 days				
19 days				
27 days				
35 days				
41 days				
77 days				
84 days				

APPENDIX B
MODEL DESCRIPTIONS

NIRS MODEL

A dynamic compartment model with selected transfer coefficients for tritium uptake by aquatic animals (as depicted in Figure B.1), was developed by K. Miyamoto of the National Institute of Radiological Sciences (NIRS) in Japan. The NIRS model was run twice (in May and November 2005) for the purposes of this scenario. The November model and calculations included an additional OBT compartment (i.e., mussel OBT-2) that was not present in the May version. The calculations were carried out using the ERMA (Environmental Radionuclide Movement Assessment) system developed by NIRS.

In applying the NIRS model, a number of assumptions were made. For example, based on the final report of the *Perch Lake Scenario* (Davis, 2005), the HTO concentration in lake water is not expected to be homogeneous, but varies with location and season. Therefore, the triplicate HTO concentration measurements that had been taken in the lake water in the vicinity of each set of cages (i.e., Cages 1 and 2; Cages 3 and 4) were averaged for each sampling time.

It was assumed that the mussels started vigorous filtration of water, suspended materials, plankton and organic matter in the water (for Cages 1 and 2), or of the sediments at the sediment-to-water interface (for Cages 3 and 4), immediately following mussel transplantation into Perch Lake. Visual observation of the transplanted mussels at the start of the experiment confirmed this assumption. In addition, it was assumed that there would be no differences in the filtration activities of the mussels exposed to water only (Cages 1 and 2) and to both water and sediments (Cages 3 and 4); however, it was assumed that a 3-fold higher concentration of sediments was ingested by mussels that had been placed at the sediment-to-water interface, compared to those that had access to the water column only. This assumption was based on the 3-fold advantage in mussel nutrition that has been reported by a fishery in Japan in sea shells of mussels cultivated at the sea bottom compared to those cultivated in cages hanging in the water column.

It was also assumed that the concentration of mussel tissue free-water tritium (TFWT) and non-exchangeable OBT (nOBT) in compartments OBT-1 and OBT-2 increased over time following transplantation. Values for the transfer coefficients were estimated through simulations using the basic model, until the mussel TFWT-to-lake water HTO ratio reached a value of 0.9 and the nOBT-to-lake water HTO ratio reached a value of 0.7, based on the findings of the Perch Lake Scenario.

No consideration was given in the NIRS model to the daily water temperatures and their potential influence on the growth of the mussels during the experiment, to the variation of weight or metabolic activity of individual mussels, or to the order of placement and sampling of the cages. The 95% confidence interval was assumed to be 5% of each endpoint.

SRA MODEL

The SRA model, which was developed by M. Saito of the Kyoto University Safety Reassurance Academy (SRA) in Japan, is a simple, two-compartment model that includes one TFWT compartment and one OBT compartment. The rate of tritium uptake into each compartment is determined using transfer coefficients, accounting for the difference in specific activity between the environment and the body of the mussel.

The SRA model assumes that the mussels assimilate TFWT from plankton, sediments and surface water, although TFWT uptake from plankton is considered negligible. In addition, mussels are assumed to incorporate OBT from plankton and sediment. Since the fractional uptake of organic hydrogen from individual nutrient sources was unknown, the uptake from plankton was assumed to occur at twice the rate from sediments.

The OBT transfer coefficient, λ , was varied in the range 0.003 to 0.0003 h⁻¹, whereas that of HTO was assumed to fall in the range 0.03 to 0.003 h⁻¹. In the report file, only the results for the case $\lambda_{\text{oht}} = 0.001 \text{ h}^{-1}$ and $\lambda_{\text{hto}} = 0.01 \text{ h}^{-1}$ were given.

Key factors determining the uncertainty of the predictions are the rates of tritium uptake from plankton, sediments and lake water. These rates can be varied when the calculations are made. The largest and smallest TFWT and OBT concentrations predicted under the above assumed range of parameter values were taken as the upper and lower limits of the confidence range.

AQUATRIT MODEL

The AQUATRIT model, which was developed by D. Galeriu of the National Institute for Physics and Nuclear Engineering-Horia Hulubei (IFIN-HH), can be applied for aquatic pathways of tritium (Galeriu et al., 2005). The model makes a number of simplifying assumptions. For example, it assumes that both water temperature and HTO concentration are spatially constant, as represented by the mean values taken at a given point in time. The model does not account for any specific attributes of the Barnes mussel but instead makes use of generic knowledge (e.g. Ren et al., 2006; Sukhotin and Portner, 2001; Wong and Levinton, 2004; Zotin and Ozernyuk, 2004). In addition, although the growth pattern for mussels is unknown, the model assumes growth patterns of quite mature animals.

The OBT concentration in aquatic animals (in Bq/kg fresh weight) is estimated using the following rate equation, where the initial condition (i.e., the initial OBT concentration in mussels) is very important in predicting OBT concentrations in the first few days following mussel transplantation:

$$\frac{dC_{org,x}}{dt} = a_x C_{f,x}(t) + b_x C_w(t) - K_{0.5,x} C_{org,x} \quad (B.1)$$

where $C_{org,x}$ is the OBT concentration in animal x (Bq/kg fresh weight);
 $C_{f,x}$ is the OBT concentration in the food of animal x (Bq/kg fresh weight);
 C_w is the HTO concentration in water (Bq/L);
 a_x is the transfer coefficient from HTO in water to OBT in animal x;
 b_x is the transfer coefficient from OBT in food to OBT in animal x; and
 $K_{0.5,x}$ is the loss rate of OBT from animal x (d^{-1}).

The tritium concentration in the dietary items consumed by a given species is calculated from:

$$C_f = \sum_{i=1}^n C_{prey,i} \cdot P_{prey,i} \frac{Dry f_{pred}}{Dry f_{prey,i}} \quad (B.2)$$

where $C_{prey,i}$ is the OBT concentration in prey species i (Bq/kg fresh weight);
 $P_{prey,i}$ is the preference for prey species i;
 $Dry f_{pred}$ is the fractional dry weight of the predatory species; and
 $Dry f_{prey,i}$ is the fractional dry weight of prey species i.

The parameters a_x and b_x in Equation B.1 reflect the metabolic regulation of tritium, as described by:

$$a_x = (1 - SAR_x) K_{0.5,x} \quad (B.3)$$

$$b_x = 0.54 \times 10^{-3} SAR_x \text{ Dry } f_x K_{0.5,x} \quad (B.4)$$

where SAR_x is the specific activity ratio when only water HTO is considered in the intake ($SAR=0.25$); and

$K_{0.5,x}$ is the sum of the relative growth rate and the mass-specific metabolic (respiration) rate, which reflects the tritium loss rate.

The model accounts for the filter-feeding behaviour of mussels, whereby food intake depends upon the water filtration rate and the food concentration in the water. In addition, there is an assimilation factor, which is quite low. Under good environmental conditions (and high food availability), the mussel will grow, and the loss rate, $K_{0.5}$, will correlate with food availability, as well as the tritium concentration in the food (as summarized in Equation B.2). Mussels can assimilate material from bacteria, plankton, detritus and dissolved organic matter. For example, the mussels in Cages 1 and 2 did not have access to detritus (sediment) in their food, but were able to consume plankton. The filtered dry matter is expected to be low for plankton, which can result in relatively slow mussel growth. By comparison, mussels in Cages 3 and 4 had access to detritus, and could, therefore, be expected to grow faster, although the average concentration of food would not higher than in Cages 1 and 2.

In the absence of specific information on *Elliptio complanata*, data reported for *Mytilis edulis* was applied in the model to estimate tritium dynamics in the transplanted mussels (e.g. Ren et al., 2006; Sukhotin and Portner, 2001; Wong and Levinton, 2004; Zotin and Ozernyuk, 2004).

Equilibration of mussel HTO with the surrounding water represents a fast process and the estimated HTO values are uncertain by a factor less than 2. However, the OBT concentration depends strongly on factors such as temperature, species and environment (growth rate), resulting in uncertainties of up to a factor 10. These uncertainties can be reduced if mussel growth rates are known.

EDF MODEL

EDF predictions are based on the OURSON model, a dynamic model that evaluates radionuclide concentrations in the aquatic and terrestrial environment resulting from liquid discharges, in order to estimate doses to humans. Consequently, only dose-relevant compartments are included in the model. In freshwater, the only aquatic animal considered is fish. The fish HTO compartment is assumed to be at equilibrium with the surrounding water,

$$A_{fish}^{HTO} = A_{eau}^{HTO} \quad (B.5)$$

where:

$$\begin{aligned} A_{fish}^{HTO} &= \text{HTO activity in fish tissue free water (Bq/L); and} \\ A_{eau}^{HTO} &= \text{HTO activity in water (Bq/L).} \end{aligned}$$

By comparison, OBT in fish is assumed to be gradually incorporated from plankton OBT, at a rate that is proportional to the feeding rate. Plankton OBT is assumed to be at equilibrium with water HTO. Thus, formation of OBT in fish is described by the following equation:

$$\frac{dA_{fish}^{OBT}(t)}{dt} = -k_{ing} A_{fish}^{OBT}(t) + k_{ing} \cdot DF_{phyto} \cdot \frac{H_{phyto}}{H_{fish}} \cdot A_{eau}^{HTO}(t) \quad (B.6)$$

where:

$$k_{ing} = \frac{I \cdot D}{W}$$

and

A_{fish}^{OBT} = OBT specific activity in fish (Bq/L combustion water);

k_{ing} = relative feeding rate (d^{-1});

I = food intake (kg dry weight d^{-1});

D = digestibility (unitless);

W = animal dry weight (kg);

DF_{phyto} = 'discrimination' factor, ratio between OBT in phytoplankton (Bq/L combustion water) and HTO in water (Bq/L);

H_{phyto} = average phytoplankton OBH in g/kg dry matter; and

H_{fish} = average fish OBH in g/kg dry matter.

Adaptation to the Mussel Scenario

First EDF Model Run (November 2005)

In a first scenario run, the OURSON fish model was directly applied to the mussel. The estimate of k_{ing} was based on a number of considerations. For example, according to the measures of biomass (from Table A.5 of the scenario), there was no visible growth of mussels during the experiment. It was, therefore, assumed that these adult mussels were in the stationary growth phase with a feeding rate corresponding to maintenance metabolism. A value of $3 \times 10^{-3} \text{ d}^{-1}$ was assigned for this parameter based on the mean value recommended for fish by Sheppard et al. (2006). In addition, DF_{phyto} was assumed to be equal to 0.7 (EMRAS Perch lake scenario report), and H_{phyto} and H_{mussel} were assigned the same value of 6% (Jorgensen, 1979).

The model was the same for the cages in the water column and those at the sediment-water interface, although the difference in location could have an influence on the filtration rate, which in turn would affect the metabolic requirements of the mussels. In addition, no consideration was given either to the daily water temperatures or their potential influence on mussel metabolism.

Second EDF Model Run (May 2006)

After the November 2005 Tritium Working Group meeting, an additional compartment was included in the model to address the under-prediction of initial OBT concentrations. This compartment represents the food particles inside the mussel (including food particles on the soft tissue surface, as well as ingested particles). Observations made for *Mytilus edulis*, a marine mussel, showed that food particles could represent as much as 30% of mussel soft tissue weight. This value was assumed to be relevant for Barnes mussels. OBT in this compartment was calculated using Equation B.6, with a turn-over rate, k_{ing} , corresponding to the particle filtration rate.

Thus, in the second EDF run, OBT in mussel (Equation B.6) was calculated according to the following equation:

$$A_{mussel}^{OBT} = 0.7 \times A_{soft-tissue}^{OBT} + 0.3 \times A_{food-particles}^{OBT} \quad (\text{B.7})$$

where $A_{soft-tissue}^{OBT}$ and $A_{food-particles}^{OBT}$ were both calculated using Equation B.6, with their own k_{ing} values. k_{ing} was set equal to 0.02 d^{-1} when calculating the activity in soft tissue. This value was derived from the metabolic requirement of *Mytilus edulis* (from Tremblay et al., 1998) and seemed more adapted to the scenario than the fish value used in the first model run. Assuming a filtration rate of 38 L/day and a suspended particle concentration in water of 10 mg/L, k_{ing} for food particles was estimated to be 0.33 d^{-1} . The predicted OBT concentrations are shown as a function of time in Figure B.2.

BIOCHEM MODEL

The BIOCHEM Model was developed by F. Baumgärtner of the Munich Technical University (TUM) in Germany. The basic premise of this model can be summarized by the following equation:

$$\text{OBT} = \text{CBT} + \text{XBT} + \text{YBT} \quad (\text{B.8})$$

where CBT is carbon-bound tritium (also referred to as non-exchangeable OBT); YBT means hydrate bound tritium (one form of exchangeable OBT); and XBT is tritium bound to oxygen, nitrogen and sulphur atoms (designated as 'X' atoms), which represents another form of exchangeable OBT.

Basically, the tritium nuclei (or tritons) of YBT and XBT are exchangeable with the hydrogen nuclei of water, but are not transferred to water during the rinsing process of OBT analysis because they are "buried" inside the biopolymers, and thus inaccessible to water during wetting (Baumgärtner and Donhär, 2004). However, in living systems "buried tritium" is exchangeable as biomolecules are formed and undergo biochemical configuration changes. The BIOCHEM model accounts for the differences in exchangeability between exchangeable OBT (i.e., YBT + XBT) and non-exchangeable OBT (CBT) that occur in living systems, so that they can be considered in tritium dose estimation. In doing so, different distribution factors are applied to distinguish between YBT and XBT in model predictions, where $\alpha_{\text{YBT}} = 1.4$ and $\alpha_{\text{XBT}} = 2.0$ (Baumgärtner and Kim, 2000). Such distribution factors have been estimated based on theoretical information and mathematical constants that have been reported by Griffiths et al. (1993), Saenger (1987), Klapper (1977) and Baumgärtner (2005), as follows:

$$(\text{H}_{\text{exch}}/\text{M})_{\text{XBT-DNA}} = 1.9 / 331 \quad (\text{B.9, from Griffiths et al., 1993});$$

$$(\text{H}_{\text{exch}}/\text{M})_{\text{YBT}} = 20 / 331 \quad (\text{B.10, from Saenger, 1987});$$

$$(\text{H}_{\text{exch}}/\text{M})_{\text{XBT-protein}} = 1.83 / 109 \quad (\text{B.11, from Klapper, 1977});$$

where H_{exch} represents exchangeable hydrogen (i.e., XBH+YBH);
M is the stoichiometric unit;
XBT-DNA is the XBT bound into DNA;
XBT-protein is the XBT bound into proteins; and

During the combustion process, the tritium in the dry matter (in Bq/kg dry weight) is converted into tritium in combustion water (in Bq/L). This conversion is accounted for using the water equivalent factor, W_{eq} (L/kg dry weight), which differs between different sample types but has a typical value of 0.58 L/kg dry weight.

Estimation of YBT and XBT

The BIOCHEM model provides a numerical estimation of ‘buried tritium’, YBT and XBT, assuming that living systems consist of proteins, DNA and carbohydrates only, since these represent the main components of any living system and may be relevant in dose calculations. Their overall molecular constitutions are approximately known and the model adopts quantitative relationships between these components. In the mussel uptake scenario calculation, it was assumed that mussels consist of proteins and DNA only, and focus was placed on formation of XBT and YBT.

The model assumes that OBT concentrations in carbohydrates are negligible in mussels (which implies that CBT represents only a small fraction of the total OBT in mussel tissues), although the justification for these assumptions is unclear.

Based on past experimental work to measure OBT concentrations in the DNA of fish sperm (Baumgärtner, 2000), it was concluded that freeze-drying of the samples might potentially lead to an under-estimation of OBT. YBT is a molecular unit, which is fundamentally volatile either at elevated temperature or at low pressure, and 24-hour freeze drying at -25°C and 10^{-6} mbar is required to separate the hydrates from fish sperm DNA; however, freeze-drying over longer time periods (e.g. 48 or 96 hours) can lead to a loss in YBT. For this reason, for the purposes of the BIOCHEM model, it was important to provide a detailed description of the experimental procedures.

Accordingly, the BIOCHEM model assumes that YBT concentrations in mussel tissues are dependent upon the number of buried hydrates in DNA (YBT_{DNA}), which comprises 10 to 12 water molecules per stoichiometric unit (Saenger, 1987), as well as the YBT retention fraction, R_{YBT} , which is much less than unity:

$$\text{YBT} = \text{YBT}_{\text{DNA}} R_{\text{YBT}} \quad (\text{B.12})$$

By comparison, XBT can be found in both DNA and protein. Thus, the maximum amount of buried tritium (or exchangeable OBT) in mussels is estimated according to the following equation:

$$\text{Maximum Exchangeable OBT} = \text{XBT}_{\text{DNA}} + \text{XBT}_{\text{protein}} + \text{YBT}_{\text{DNA}} R_{\text{YBT}} \quad (\text{B.13})$$

In the “non-buried” state, an equilibrium of exchange was assumed to exist between the hydrogen isotopes of water and X-bound hydrogen (i.e., exchangeable hydrogen, XBH) in any organic compartment, i , according to the following equation:

$$\frac{T_i}{\text{XBH}_i} = \alpha_{\text{XBT}, i} \cdot \frac{T_{\text{aqua}}}{\text{XBH}_{\text{aqua}}} \quad (\text{B.14})$$

where T_i = total tritium concentration in the organic compartments of the mussel (Bq/kg fresh weight);

XBH_i = amount of X-bound hydrogen in the mussel organic compartments;

$\alpha_{\text{XBT},i}$ = XBT distribution factor for mussel organic compartment, i , (assumed to equal 2.0);

T_{aqua} = total tritium concentration in the mussel water compartment (Bq/kg fresh weight); and

XBH_{aqua} = amount of X-bound hydrogen in mussel water.

Equation B.14 is equivalent to:

$$\frac{T_i \cdot M}{\text{XBH}_i} = \alpha_{\text{XBT},i} \cdot \frac{M \cdot T_{\text{aqua}}}{\text{XBH}_{\text{aqua}}} = \alpha_{\text{XBT},i} \cdot \frac{M_{\text{oxygen}} \cdot T_{\text{aqua}}}{M_{(2 \cdot \text{hydrogen})}} = \alpha_{\text{XBT},i} \cdot \frac{18 \cdot T_{\text{aqua}}}{2} \quad (\text{B.15})$$

where XBH is the number of X-bound hydrogen atoms per atomic mass number of the stoichiometric unit, M ;

M_{oxygen} is the molar mass of an oxygen atom (18 g/mol), since there is one oxygen atom per water molecule; and

$M_{(2 \cdot \text{hydrogen})}$ is molar mass of two hydrogen atoms (2 g/mol), since there are two hydrogen atoms per water molecule.

If T_{aqua} is represented by HTO and the contributions of all compartments are summed up, (assuming the nucleotide ratios of different compartments are equal and that CBT is negligible), Equation B.15 can be converted to Equation B.16, as follows:

$$(\text{XBT} + \text{YBT}) = \left(\frac{9 \cdot [\text{HTO}]}{\text{Weq} \cdot \left\{ \sum_i \alpha_{\text{XBT}} \cdot \left(\frac{H_{\text{exch},i}}{M_i} \right) \cdot m_i \right\}} \right) + \alpha_{\text{YBT}} \cdot \left(\frac{H_{\text{exch},\text{YBT}}}{M_{\text{YBT}}} \right) \cdot R_{\text{YBT}} \cdot \frac{m_{\text{DNA}}}{m_{\text{total}}} \quad (\text{B.16})$$

where $(\text{XBT}+\text{YBT})$ is the OBT concentration in mussel tissue (Bq/L);

m_i is the mass of compartment i (kg);

m_{DNA} is the mass of DNA in the mussel tissue (kg); and

m_{total} is the total mass of the mussel tissue (kg).

Despite these findings, however, it is important to note that for other types of samples and tissues (such as plant leaves), such decreases in OBT may not be detectable over several days of freeze-drying.

Dynamics of YBT and XBT Formation

During formation and, to a lesser extent, during metabolic activity of biopolymers, X-bound hydrogen (XBH) atoms and neighbouring water molecules are assumed to rapidly exchange their hydrogen nuclei, at a rate that is influenced by the mass of a given hydrogen isotope (Eigen, 1963). Consequently, (YBT+XBT) formation in the transplanted mussels was assumed to proceed spontaneously with the ingress of Perch Lake water into mussel cells. The penetration of HTO into the cells was assumed to

occur by diffusion and over a very few days following a linear time scale. Additionally, the BIOCHEM model assumed that the isotope mass effect, which was expected to occur in plankton, would also occur during transfer of HTO into mussel tissue, where:

$$\text{TFWT} / [\text{HTO}] = 0.99 \quad (\text{B.17})$$

The mussels were near the end of their life span and therefore showed no significant growth in fresh weight between the time of transplantation and mussel harvest (as discussed in Section 3.2.3 above). Therefore, no additional OBT increase beyond (YBT+XBT) formation was taken into account in the model and molecular OBT exchange with plankton was considered negligible (although this may not be a realistic assumption).

Sensitivity Analysis and Parameter Definition for the BIOCHEM Model

A sensitivity analysis was carried out to test the potential influence on mussel OBT concentrations of mussel protein and DNA content, the rate of ingress of free-water tritium (FWT) into mussel cells, and the YBT retention factor (Figures B.3 to B.8). The conditions used in the analysis are summarized in Table B.1. It was concluded that FWT ingress into mussel cells was complete after 2 days, that mussel tissues consist of 75% protein and 25% DNA, and that the YBT retention factor, R_{YBT} , is 0.2.

Temporal Trends in HTO Relative to Perch Lake Water Temperature

The HTO values in both cages showed fluctuations, even over relatively short time intervals and distances (Table 3), as did Perch Lake water temperature (Table A.1). The relationship between mean monthly surface water temperature and time (in months) was summarized as follows for use in the BIOCHEM model (Figure B.9):

$$T[^\circ\text{C}] = (\text{mean}T_{\text{July}} - \text{mean}T_{\text{October}}) \exp(-\ln 2 \cdot t / \tau) + \text{mean}T_{\text{October}} \quad (\text{B.18})$$

where $T[^\circ\text{C}]$ is the Perch Lake surface water temperature;
 $\text{mean}T_{\text{July}}$ is the mean water temperature in July;
 $\text{mean}T_{\text{October}}$ is the mean water temperature in October;
 t is time (d); and
 τ is the radiological half-life (d).

Although a similar modelling approach could theoretically be applied to approximate temporal changes in Perch Lake HTO concentrations, very large deviations in HTO were predicted based on measured data. Therefore, arithmetic mean Perch Lake HTO concentrations were used in the model to predict (XBT+YBT) in the mussels, such that mussel OBT fluctuations were proportional to the mean HTO water concentrations.

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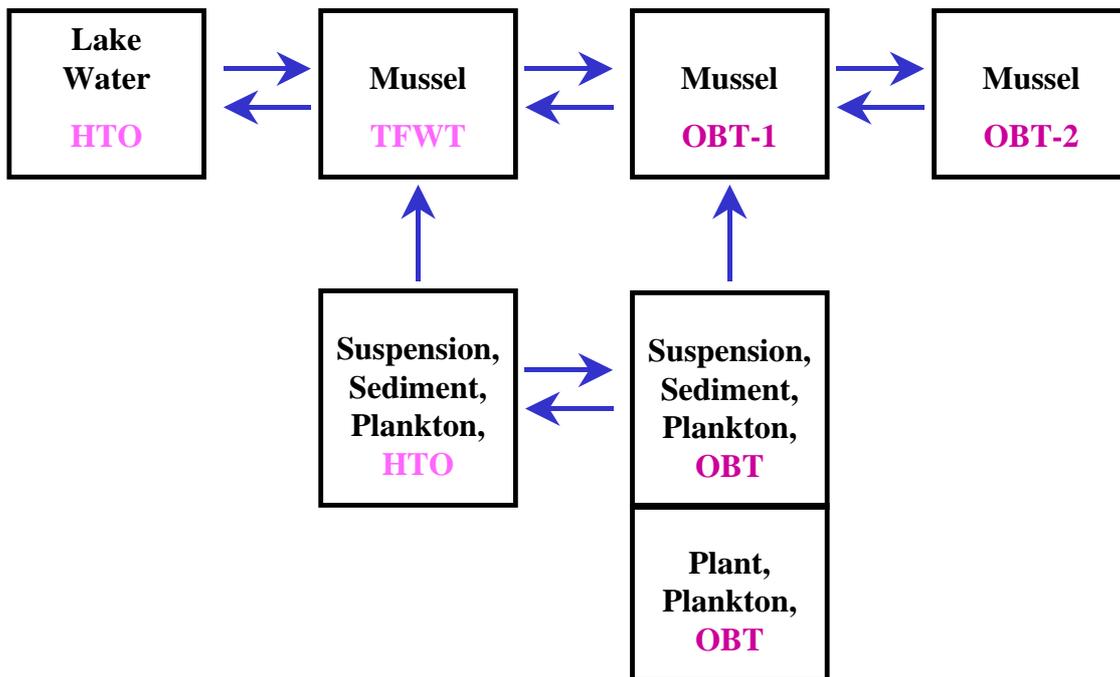


Figure B.1: Conceptual model depicting compartments and the linkages between compartments, as assumed in the NIRS model (Japan).

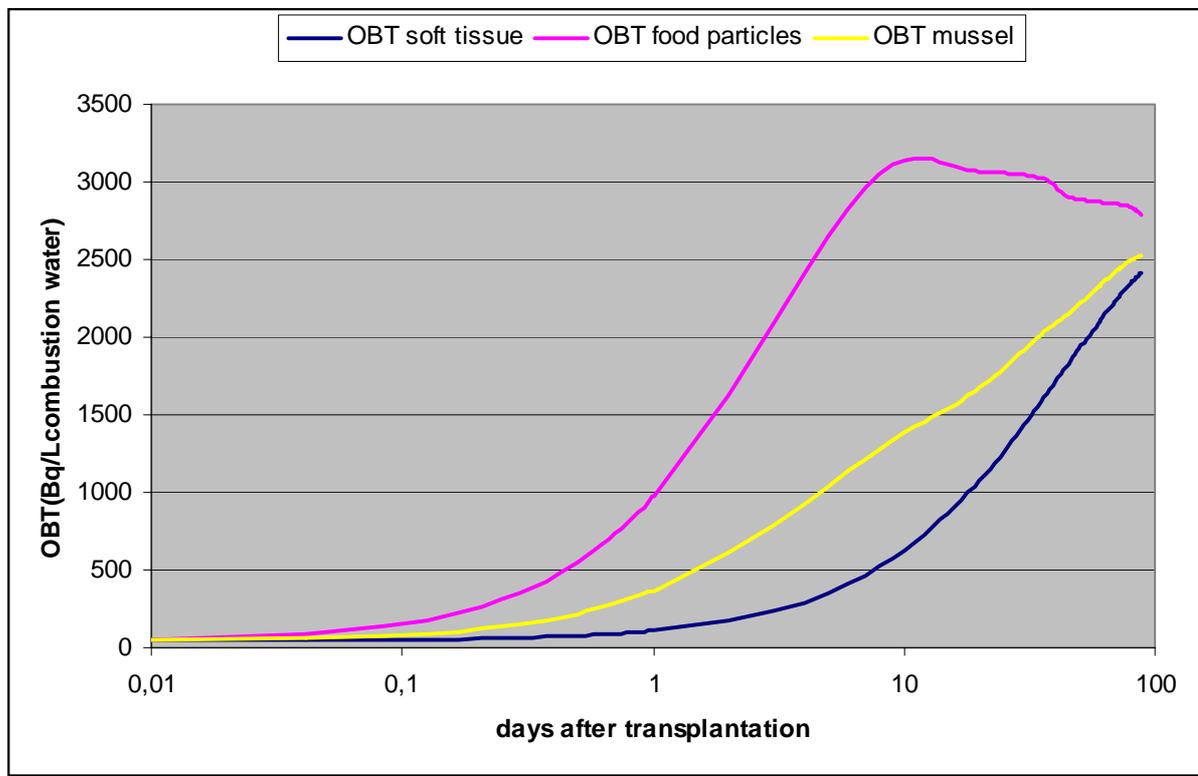


Figure B.2: EDF predictions of OBT in mussels of Cages 1 and 2.

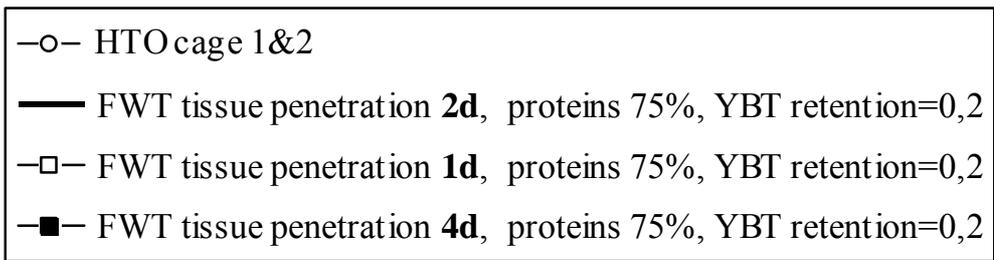
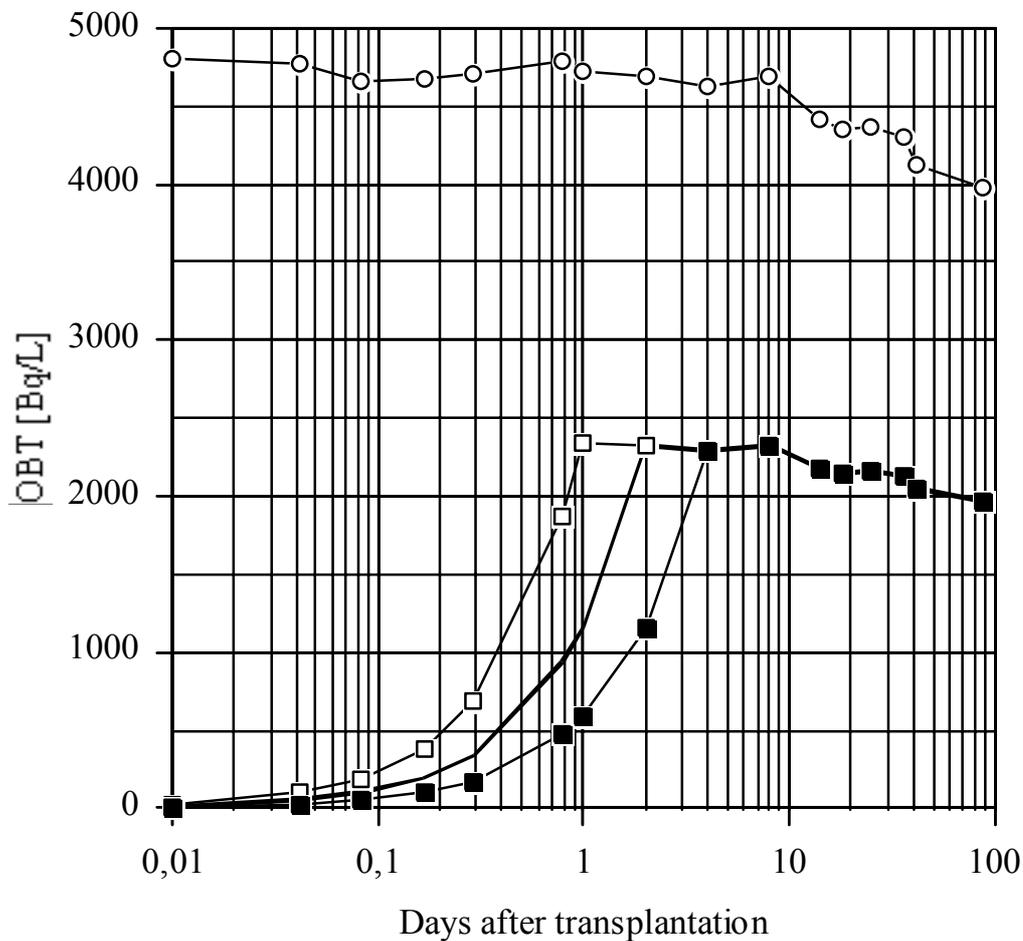


Figure B.3: BIOCHEM model predictions of temporal changes in Cage 1 and 2 mussel tissue OBt concentrations relative to changes in Perch Lake water HTO levels under conditions of varying free-water tritium (FWT) tissue penetration times. Protein levels of 75% and a YBT retention of 0.2 were assumed.

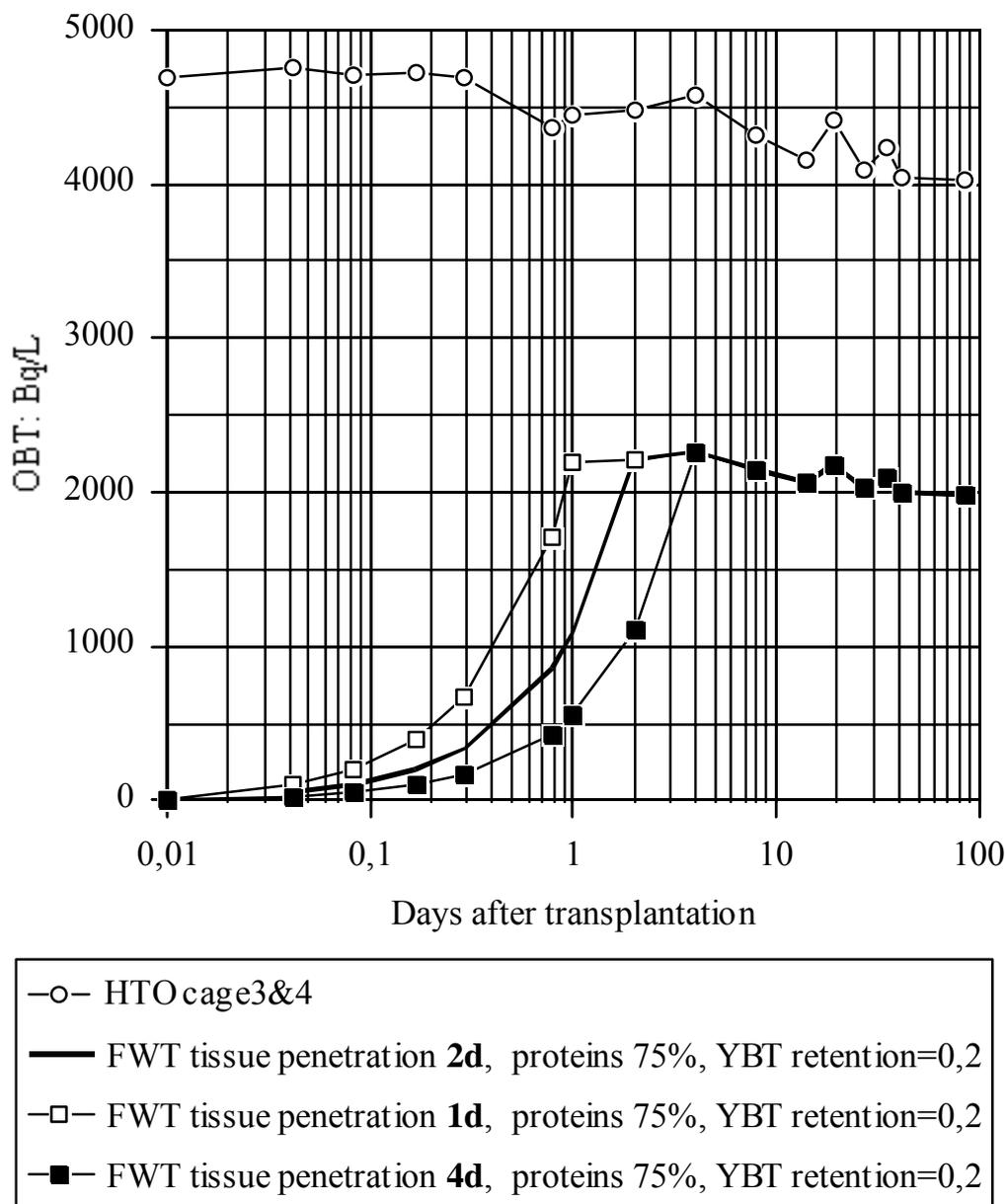


Figure B.4: BIOCHEM model predictions of temporal changes in Cage 3 and 4 mussel tissue OBt concentrations relative to changes in Perch Lake water HTO levels under conditions of varying free-water tritium (FWT) tissue penetration times. Protein levels of 75% and a YBT retention of 0.2 were assumed.

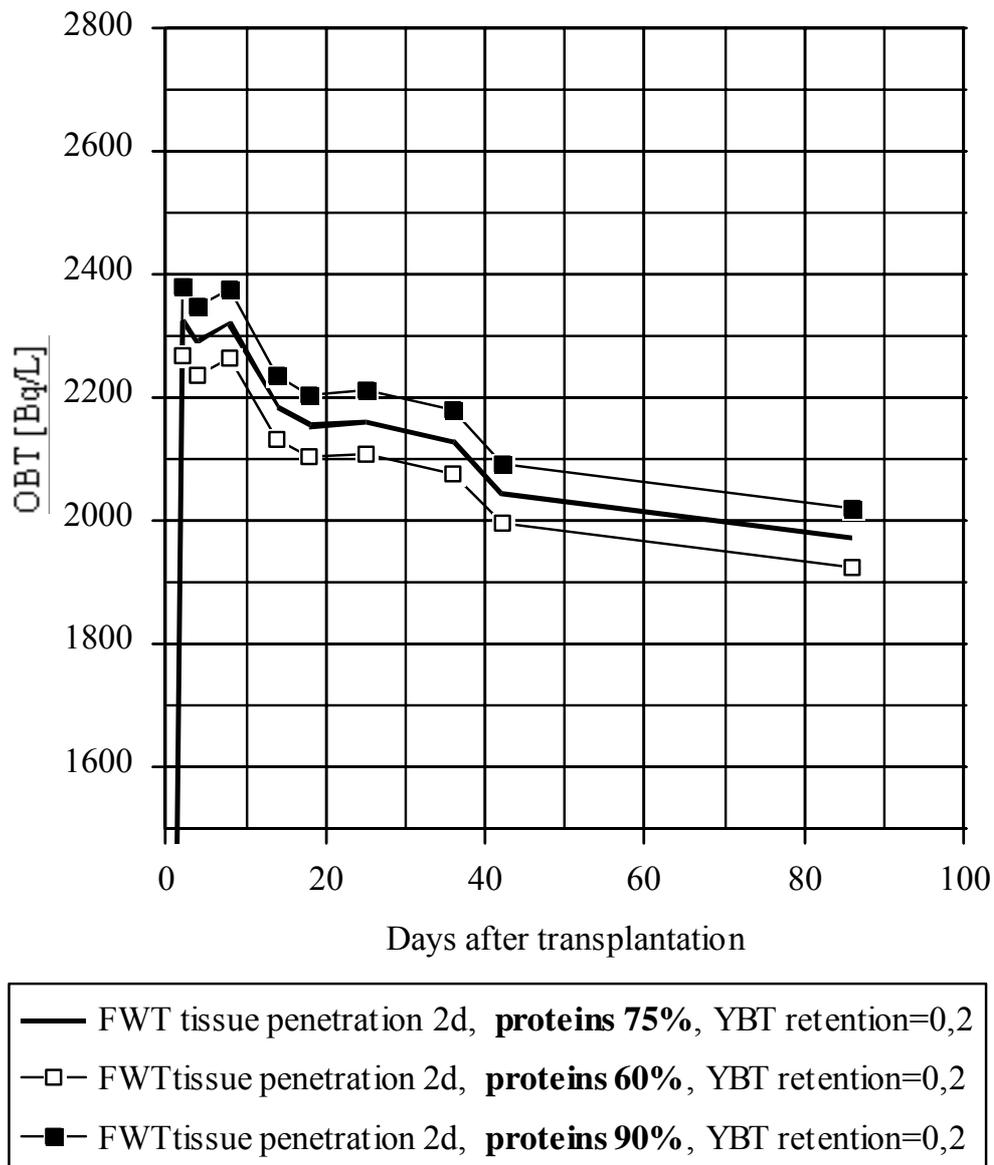


Figure B.5: BIOCHEM model predictions of temporal changes in Cage 1 and 2 mussel tissue OBt concentrations relative to changes in Perch Lake water HTO levels under conditions of varying mussel protein content. Free-water tritium (FWT) tissue penetration times of 2 days and a YBT retention of 0.2 were assumed.

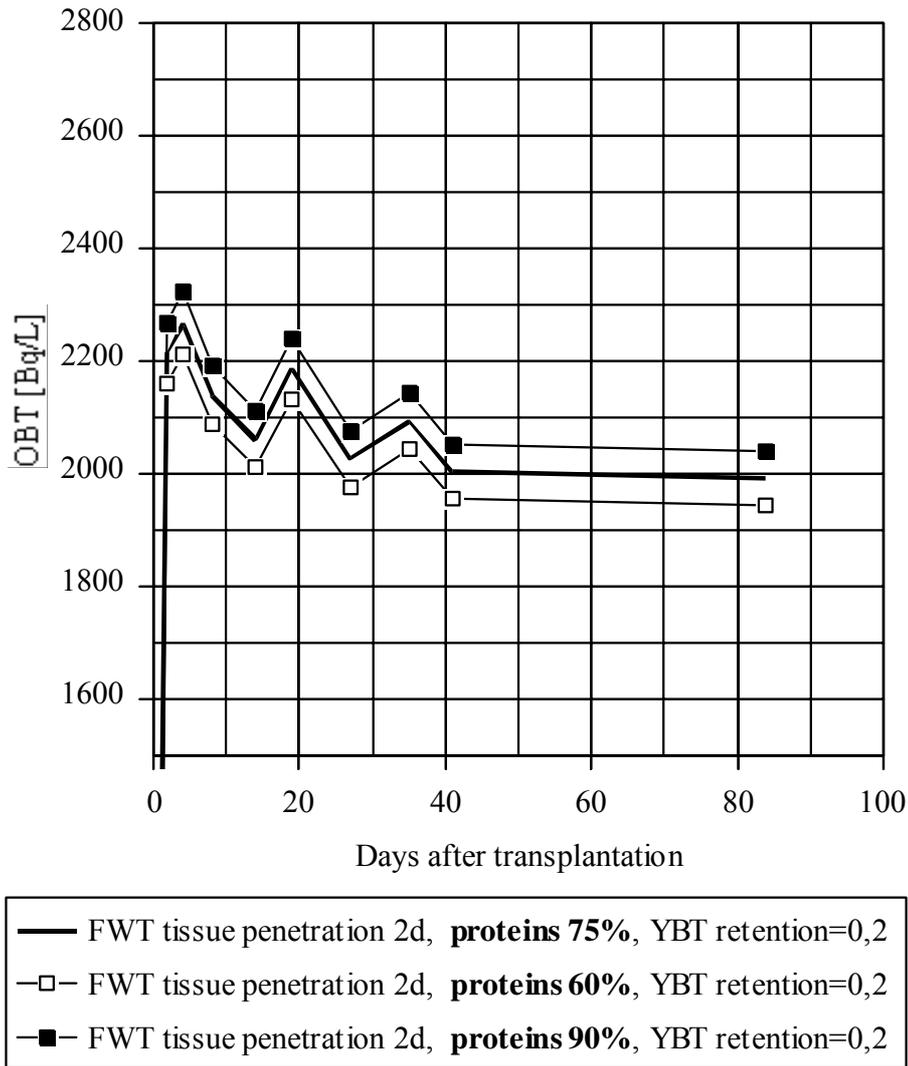


Figure B.6: BIOCHEM model predictions of temporal changes in Cage 3 and 4 mussel tissue OBt concentrations relative to changes in Perch Lake water HTO levels under conditions of varying mussel protein content. Free-water tritium (FWT) tissue penetration times of 2 days and a YBT retention of 0.2 were assumed.

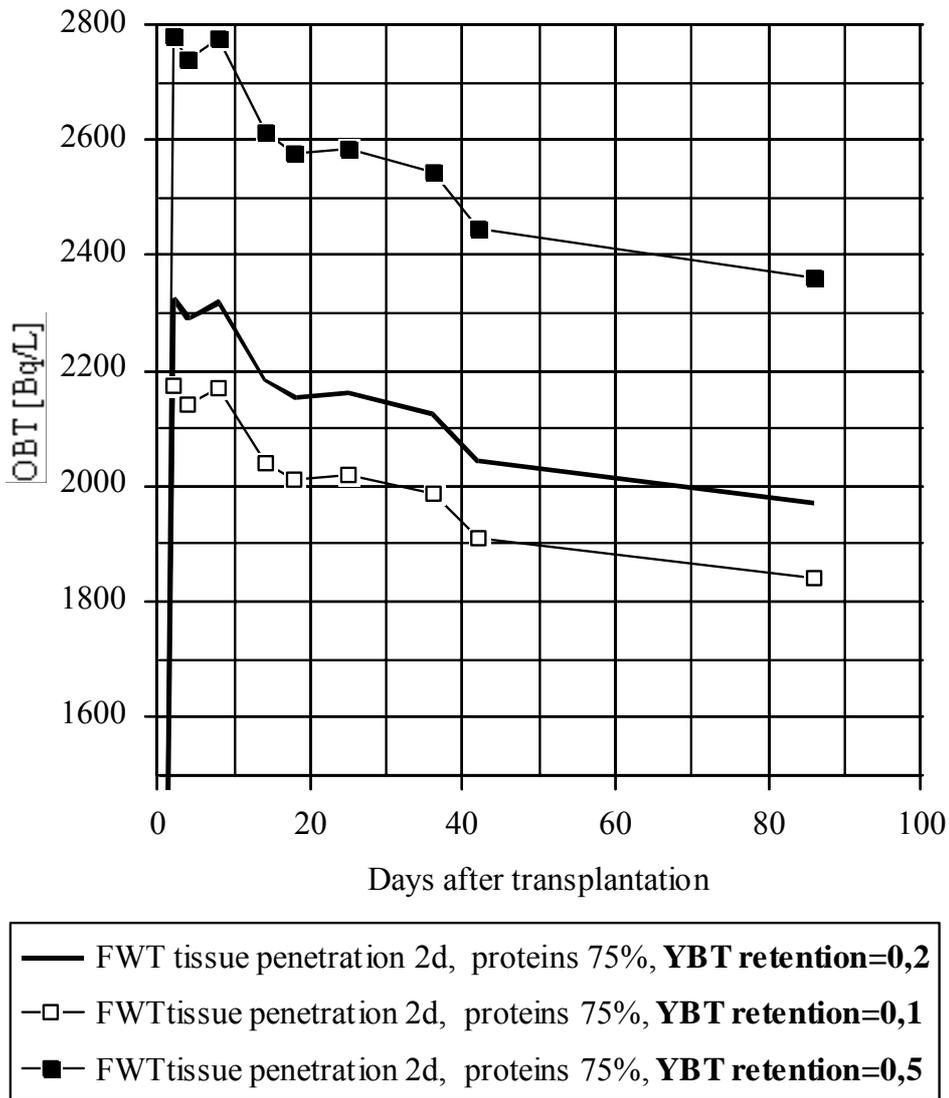


Figure B.7: BIOCHEM model predictions of temporal changes in Cage 1 and 2 mussel tissue OBt concentrations relative to changes in Perch Lake water HTO levels under conditions of varying YBT retention. Free-water tritium (FWT) tissue penetration times of 2 days and a mussel protein content of 75% (with a DNA content of 25%) were assumed.

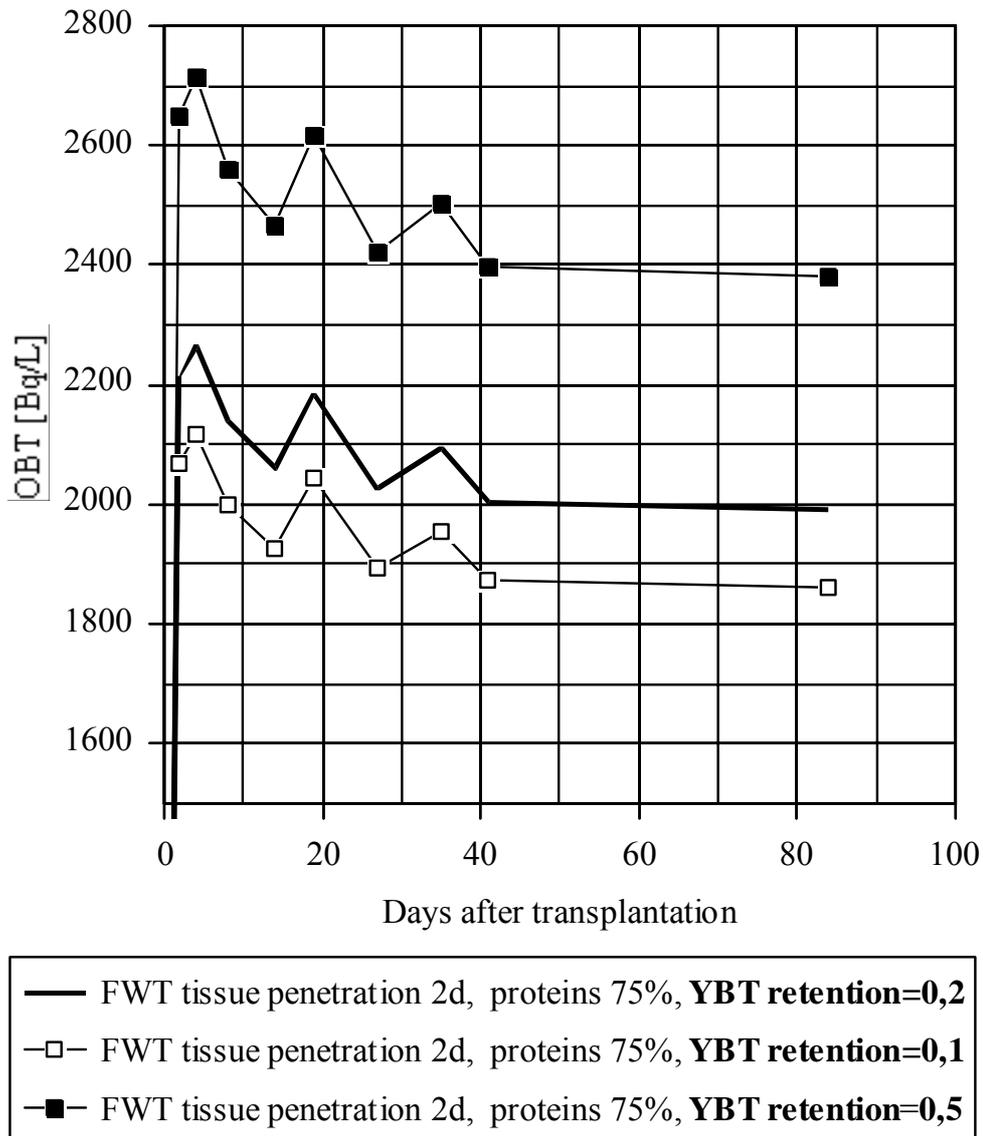


Figure B.8: BIOCHEM model predictions of temporal changes in Cage 3 and 4 mussel tissue OBt concentrations relative to changes in Perch Lake water HTO levels under conditions of varying YBT retention. Free-water tritium (FWT) tissue penetration times of 2 days and a mussel protein content of 75% (with a DNA content of 25%) were assumed.

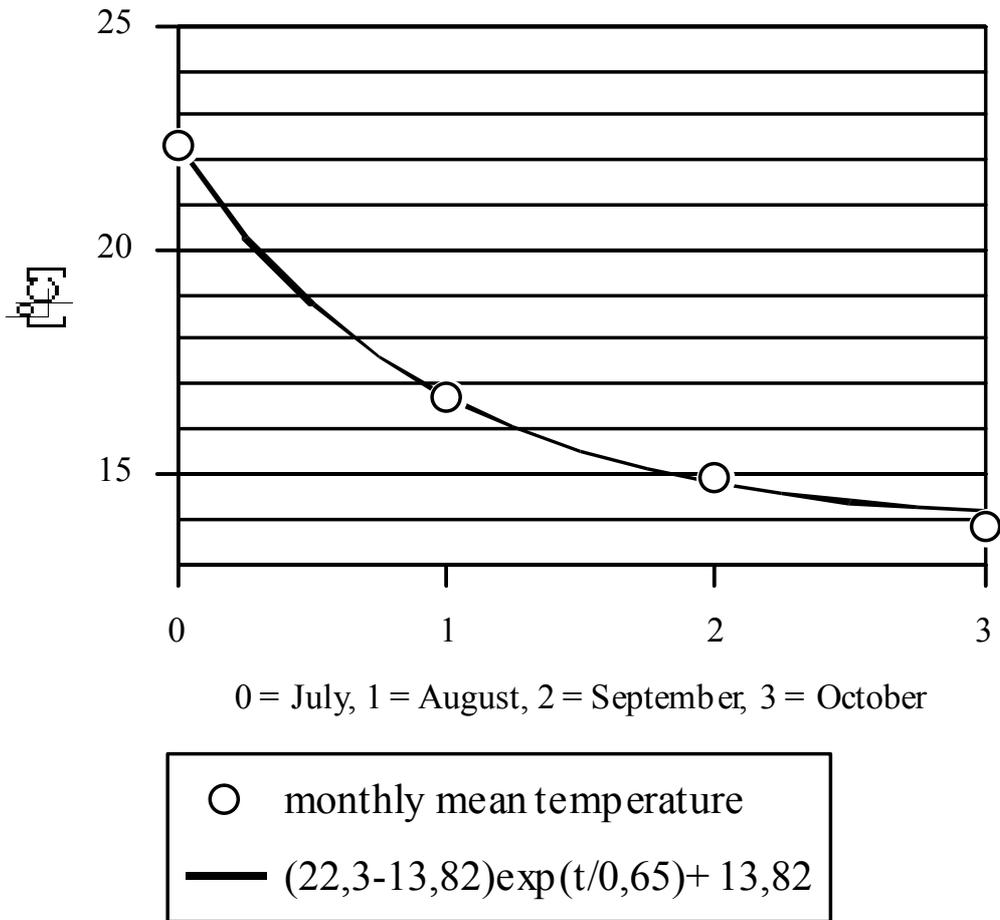


Figure B.9: Approximation of mean monthly Perch Lake water temperature (estimated by F. Baumgärtner).

Table B.1: Summary of BIOCHEM model test conditions for sensitivity analysis to determine the potential influence of mussel percent protein content, mussel percent DNA content, rate of ingress of free-water tritium (FWT) into mussel cells and the YBT retention factor on predicted OBT concentrations in mussel tissue.

Scenario No.	Figure No.	FWT Tissue Ingress (days)	% Protein	% DNA	YBT Retention, R_{YBT}
1. a.	B.3 and B.4	2	75%	25%	0.2
1. b.	B.3 and B.4	1	75%	25%	0.2
1. c.	B.3 and B.4	4	75%	25%	0.2
2. a.	B.5 and B.6	2	75%	25%	0.2
2. b.	B.5 and B.6	2	60%	40%	0.2
2. c.	B.5 and B.6	2	90%	10%	0.2
3. a.	B.7 and B.8	2	75%	25%	0.2
3. b.	B.7 and B.8	2	75%	25%	0.1
3. c.	B.7 and B.8	2	75%	25%	0.5