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TEMPERATURE INFLUENCES ON WATER PERMEABILITY AND CHLORPYRIFOS UPTAKE IN AQUATIC INSECTS WITH DIFFERING RESPIRATORY STRATEGIES

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Abstract—Aquatic insects have evolved diverse respiratory strategies that range from breathing atmospheric air to breathing dissolved oxygen. These strategies result in vast morphological differences among taxa in terms of exchange epithelial surface areas that are in direct contact with the surrounding water that, in turn, affect physiological processes. This paper examines the effects of acute temperature shifts on water permeability and chlorpyrifos uptake in aquatic insects with different respiratory strategies. While considerable differences existed in water permeability among the species tested, acute temperature shifts raised water influx rates similarly in air-breathing and gill-bearing taxa. This contrasts significantly with temperature-shift effects on chlorpyrifos uptake. Temperature shifts of 4.5°C increased ^{14}C-chlorpyrifos accumulation rates in the gill-bearing mayfly *Cinygma* sp. and in the air-breathing hemipteran *Sigara washingtonensis*. However, the temperature-induced increase in ^{14}C-chlorpyrifos uptake after 8 h of exposure was 2.75-fold higher in *Cinygma* than in *Sigara*. Uptake of ^{14}C-chlorpyrifos was uniformly higher in *Cinygma* than in *Sigara* in all experiments. These findings suggest that organisms with relatively large exchange epithelial surface areas are potentially more vulnerable to both osmoregulatory distress as well as contaminant accumulation. Temperature increases appear more likely to impact organisms that have relatively large exchange epithelial surface areas, both as an individual stressor and in combination with additional stressors such as contaminants.

Keywords—Aquatic insects   Temperature   Respiratory strategy   Permeability   Chlorpyrifos

INTRODUCTION

Aquatic insects play fundamental roles in freshwater ecosystems. They are important food sources for fish and birds and play significant roles in nutrient cycling and organic materials processing [1]. Because of their ecological importance and diversity, aquatic insects are used extensively to evaluate ecosystem health and water quality through field biomonitoring [2,3] and to a lesser extent through laboratory bioassays. Ecologists have observed that certain taxa tend to be extirpated from systems with degraded water quality. Similarly, toxicologists have observed marked sensitivity differences among aquatic insect species [4,5].

Despite the widespread utilization of insects to evaluate environmental quality, life history characteristics that relate to differences in species' responses to environmental stressors have received surprisingly little attention [6]. To date, functional feeding morphology [7] has been the primary life history characteristic explored in relation to degraded ecological conditions and has been most useful in assessing streams with severely altered riparian habitats. However, few diagnostic tools currently exist to evaluate insect community-level responses to water chemistry–associated stressors such as temperature or chemical contamination. Other life history and physiological characteristics are important determinants of species responses to degraded water chemistry conditions and merit investigation.

The small size of aquatic insects results in an extremely high surface-to-volume ratio, which in turn requires that the integument provide a substantial barrier to water and ions. Osmotic gradients favor the passive loss of ions as well as the influx of water [8–10]. This osmoregulatory situation is generally countered by barriers of waterproofing lipids, waxes, and proteins on the integument and/or by compensatory activity of chloride cells on the body surface (Fig. 1). Insects are extremely heterogeneous with respect to the integument both in terms of structure and function. This is, in part, because of their interesting evolutionary history as secondarily aquatic organisms, arising from numerous invasions of freshwater habitats [11]. Integument differences among taxa partially result from a wide variety of respiratory strategies that range from breathing atmospheric air to breathing dissolved oxygen in water through exchange epithelia [12]. In this paper, we use the term ''exchange epithelium'' specifically to describe a thin layer of cells that are effectively in immediate physiological contact with the surrounding water.

Insect respiratory characteristics have received surprisingly little attention, particularly since insects are used so frequently to assess water quality [13]. Studies have demonstrated the importance of biological barriers in terms of contaminant uptake mechanisms and fluxes in aquatic insects [14–16]. Recent findings demonstrate substantial differences among species in terms of water permeability and chlorpyrifos (an organophosphate insecticide) uptake rates. Highly water-permeable insects (dissolved oxygen breathers) have higher chlorpyrifos uptake rates than slightly water-permeable insects (air breathers) [17]. These differences are likely based on vast differences in physiological interaction or connectivity with the surrounding water via exchange epithelial surfaces as well as body size. It would be useful to further evaluate the importance of exchange epithelial surfaces in determining temperature-modulated changes in both water influx and chlorpyrifos accumulation.

Elevated temperatures pose major problems to aquatic or-

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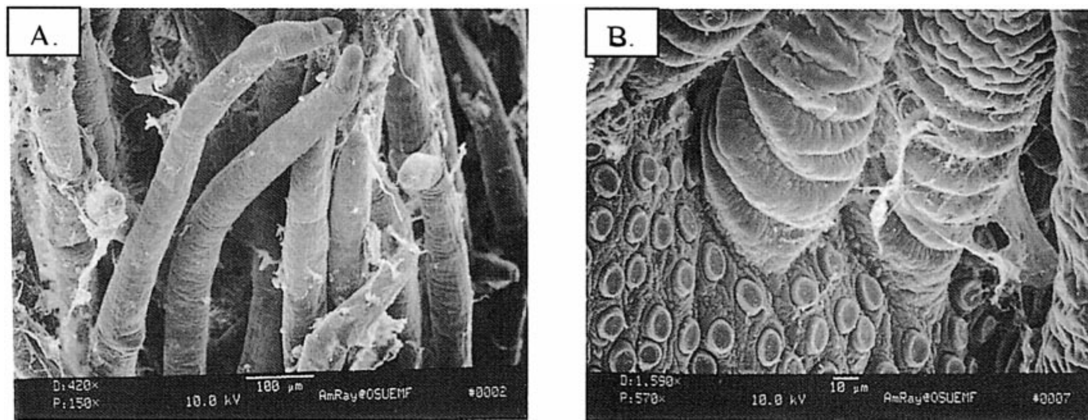

Fig. 1. Electron micrographs of a single *Pteronarcys californica* gill tuft. (**A**) The extensive epithelial surface area of the gills; (**B**) chloride cells, which densely populate the basal structure of the gill.

ganisms. For example, a significant number of Oregon water bodies that are designated as water quality limited under section 303d of the Clean Water Act (http://www.epa.gov/region5/water/cwa.htm) are listed because of elevated temperatures. Research with fish suggests that elevated temperatures may result in morphological changes in gill epithelia [18] and osmoregulatory stress [19,20]. Elevated temperatures have also been shown to reduce aquatic insect growth and fecundity in mayflies [21,22] and stoneflies (D. Buchwalter, unpublished data). However, few physiological studies with insects assess the role that respiratory system morphology plays in determining temperature responses across species. The decrease in oxygen solubility at warmer temperatures would suggest that water breathing might be disadvantageous in warming conditions. We hypothesize that water permeability, which is mediated by body size and exchange epithelial surface area, could also be an important determinant of temperature sensitivity differences among aquatic insect taxa.

Chlorpyrifos is a widely used, highly toxic organophosphate insecticide. It can run off to surface waters and create water quality problems in areas of heavy use, such as the San Juaquin River in California, USA. Its lipophilic nature (K_{ow} = 31,000) allows the compound to sorb onto the integument of aquatic insects and diffuse across plasma membranes. The rate at which chlorpyrifos is accumulated in aquatic insects is influenced by body size and exchange epithelial surface areas [17].

This paper examines the temperature modulation of ^3H_2O permeability and ^{14}C-chlorpyrifos uptake rates in aquatic insect taxa with different respiratory strategies. The influences of acute temperature shifts and acclimation on water influx rates are examined in larvae of the plecopteran *Pteronarcys californica* (Nelson). For comparative purposes, temperature-modulated ^3H_2O influxes in the hemipteran *Notonecta kirvyi* (Hungerford) and trichopteran *Dicosmoecus gilvipes* (McLachlan) are also examined. A second study examines temperature effects on water and chlorpyrifos uptake in the ephemeropteran *Cinygma* sp. and hemipteran *Sigara washingtonensis* (Hungerford).

MATERIALS AND METHODS

Insects: Collecting and handling

All larvae were collected with a D-frame kick net and transported in wet moss in ice-filled coolers. Larvae of the stonefly *P. californica* were collected from the Deschutes River near Warm Springs (OR, USA). Larvae of the caddisfly *D. gilvipes* were collected from the North Fork of the Alsea River near Alsea (OR, USA). Larvae of the hemipteran *N. kirvyi* were collected from the E.E. Wilson Wildlife Refuge in Adair (OR, USA). Larvae of the mayfly *Cinygma* sp. were collected from the South Santiam River at Cascadia State Park (OR, USA). Larvae of the hemipteran *S. washingtonensis* were collected from the Middle Fork of the Willamette River directly below the Hills Creek Dam near Oakridge (OR, USA).

All insects were held in recirculating aquaria containing approximately 75 gal of soft well water collected from the U.S. Environmental Protection Agency's Willamette Research Station in Corvallis (OR, USA). Insects were acclimatized for at least 7 d prior to initiating experiments in temperature-controlled environmental chambers maintained at ±0.5°C. Conditions included a 16:8-h light:dark photoperiod with indirect full-spectrum lighting. With the exception of one set of experiments, all insects were acclimated to 8.5°C. Insects were fed ad libitum a diet consisting of a mixture of wheat, alfalfa, yeast, and Baby fish food L (TetraMin®, Melle, Germany).

Water permeability studies were conducted with five taxa and included time course studies with the plecopteran *P. californica*, hemipterans *N. kirvyi*, and *S. washingtonensis*, and ephemeropteran *Cynigma* sp. For comparative purposes, water influx studies at a single discrete time point were also conducted with *P. californica*, *N. kirvyi*, and the trichopteran *D. gilvipes*.

Water permeability studies in P. californica

Reconstituted water (0.67 mM $CaCl_2$, 0.3 mM $MgSO_4$, 1.2 mM $NaHCO_3$, and 0.5 mM KH_2PO_4) (Fisher Scientific®, Pittsburgh, PA, USA) was used for all experiments. Twenty milliliters of water were added to a 50-ml Erlenmeyer flask that contained 20 µl ^3H_2O to achieve a final activity of approximately 0.45 µCi/ml. The initial ^3H_2O activity of each bath was verified by taking two 20-µl samples, which were analyzed by a Beckman LS6800 liquid scintillation counter (Fullerton, CA, USA) with 43% ^3H counting efficiency.

Individual larvae were placed into flasks for a set duration, removed, rinsed with deionized water, blotted dry, and weighed. After weighing, the larvae were rinsed again with deionized water and blotted dry. Hemolymph was extracted with 10-µl-calibrated capillary tubes after a small incision was made at the base of the most caudal gill tuft. Hemolymph was

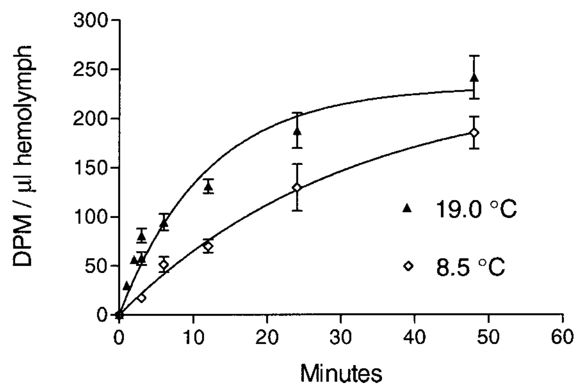

Fig. 2. The influence of temperature on ^3H_2O accumulation (measured as disintegrations per minute [dpm]) in the hemolymph of *Pteronarcys californica*. Based on these initial experiments, subsequent experiments focused on time points in the initial linear portions of these curves. Error bars represent the standard errors of the means for each time point.

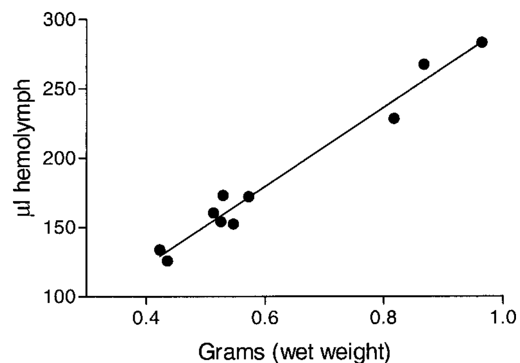

Fig. 4. ^{14}C-Inulin carboxyl was injected in the dorsal aorta to determine hemolymph volumes in *Pteronarcys californica*. Each point represents the average of at least four hemolymph samples taken between 3-min intervals after a 30-min mixing interval. This calibration curve ($r^2 = 0.97$, $p < 0.01$) was used to convert hemolymph ^3H_2O data to water influx rates.

expelled directly into scintillation vials containing 3.5-ml ScintiSafe Econo2® cocktail (Fisher).

Preliminary experiments were conducted with 8.5°C-acclimated *Pteronarcys* to determine the overall shape of the time-^3H_2O accumulation curves and allow us to focus on time points where efflux and changes in the external ^3H_2O activity were negligible (the linear portion of the uptake curves shown in Fig. 2). Subsequent experiments with 8.5°C-acclimated *Pteronarcys* were performed to examine the effects that temperature increases of 4.5 and 10.5°C had on water uptake rates (Fig. 3). A total of 46 organisms were acclimated and exposed to 8.5°C for 3, 4.5, 6, 7.5, and 12 min, respectively. A total of 48 organisms acclimated to 8.5°C were shifted to 13°C for 0.5, 0.75 1.5, 2.5, 3.5, and 4.5 min, respectively.

In other experiments, 20 organisms were acclimated and exposed to 13°C for 1.5, 2.5, 3.5, and 4.5 min, respectively, and exposed to tritiated water at acclimation temperature. In all experiments, three to six individuals were exposed to each of several time points to obtain water influx rates. A total of 166 *Pteronarcys* larvae were used in these experiments, ranging in wet weight from 0.45 to 1.89 g, with an average wet weight of 1.02 g. To convert hemolymph ^3H_2O activities to flux rates, it was essential to determine hemolymph volume.

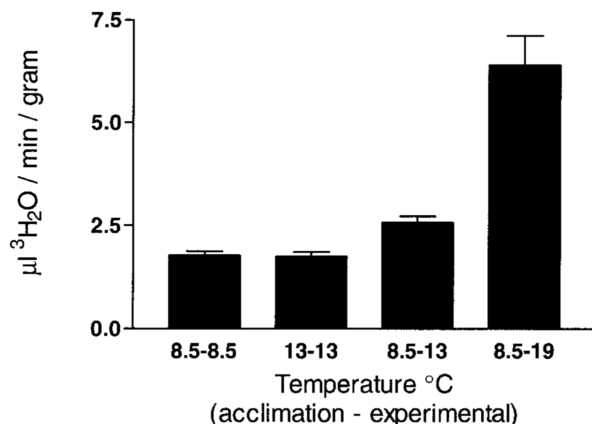

Fig. 3. Effects of temperature shifts and acclimation on water influx rate (measured as disintegrations per minute). Bars represent the slopes of linear regression models fit to uptake data. No difference was observed in influx rates for animals exposed to ^3H_2O at acclimation temperatures of 8.5 and 13°C (unpaired *t* test, $p = 0.85$). A 4.5°C shift (8.5–13) results in a 45% increase in water influx rate. A 10.5°C shift (8.5–19) results in a 3.6-fold increase in initial water influx rates (one-way analysis of variance, $p < 0.01$). Error bars represent standard errors of the slopes of uptake curves.

Determination of hemolymph volume

Hemolymph volumes were measured in *P. californica* via the injection of ^{14}C-inulin carboxy (Moravek®, Brea, CA, USA). This large sugar distributes to extracellular space and remains in circulation for hours before levels decrease through excretion [23,24]. Inulin carboxy (50 µCi) was obtained as a dry solid and dissolved in 10 ml of deionized water. Individual insects were blotted dry, weighed, and anesthetized with CO_2. They were then injected with 5.8 µl (29 *n*Ci) of inulin in the dorsal aorta with calibrated, flame-pulled 10-µl capillary tube. On removal of the pipette, any fluid emerging from the injection point was swiped with a Kim-wipe® (Kimberly-Clark, Mississauga, ON, Canada) and assayed by a liquid scintillation counter to ensure that no inulin loss occurred. Immediately after injection, the insects were placed into aluminum weighing dishes with just enough water to keep the gills moist but not enough water to disturb the injection site. Insects typically recovered from the anesthetic 5 to 8 min after injection with inulin. Following a 30-min mixing period, at least four 5-µl hemolymph samples were taken every 3 min to ensure that the inulin was thoroughly mixed. These results (Fig. 4) were used to convert hemolymph ^3H_2O counts to water influx rates with the following equation:

$$V_h = (V_s(C_o)/C_b) - V_I$$

where

V_h = hemolymph volume
V_s = volume of hemolymph sample
C_o = count of original injection
V_I = µl of solution injected
C_b = count of hemolymph sample

Water permeability studies in N. kirvyi *and* D. gilvipes

For comparative purposes, ^3H_2O uptake was examined via time course studies with 8.5°C-acclimated *N. kirvyi*. These studies were performed over short-term durations (up to 9 min), similar to *Pteronarcys*. A total of 22 larvae ranging in wet weight from 0.09 to 0.12 g, with an average wet weight of 0.11 g, were used to obtain ^3H_2O uptake rates. In a separate

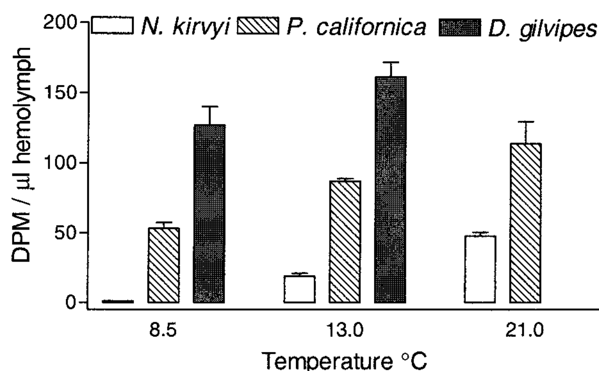

Fig. 5. The influence of respiratory strategy, temperature, and size on ^3H_2O accumulation in three species of aquatic insects (measured as disintegrations per minute [dpm]). The air-breathing *Notonecta kirvyi* was essentially impermeable to water in time course studies to 9 min at 8.5°C (data not shown). *Notonecta kirvyi* accumulated significantly less ^3H_2O at 13°C than gill-bearing species *Pteronarcys californica* and *Dicosmoecus gilvipes*. Because of the relative size differences between the large *P. californica* (1.02 g) and the smaller *D. gilvipes* (0.14 g) and *N. kirvyi* (0.11 g), exact comparisons cannot be made without hemolymph volume data. We can conclude, however, that gill-bearing species are more water permeable than air-breathing species.

experiment, three larvae each were exposed to ^3H_2O for 15 min at 12.5°C, and three larvae each were exposed to ^3H_2O for 15 min at 21°C (Fig. 5). Hemolymph was extracted from an incision at the base of the rostrum with 5-µl-calibrated capillary tubes. To address the issue of size and further explore the relationship between respiratory surface and water permeability, we collected larvae of the trichopteran *D. gilvipes* that were only slightly larger than *N. kirvyi*. *Dicosmoecus gilvipes* larvae averaging 0.14 g wet weight (range 0.09–0.19 g) were acclimated at 8.5°C. Four larvae each were exposed to ^3H_2O for 15 min at acclimation temperature, and four larvae were exposed to ^3H_2O for 15 min at 12.5°C (Fig. 5). Hemolymph was extracted with 10-µl-calibrated capillary tubes after a small incision was made at the base of the most caudal gill tuft. In a separate experiment, *Pteronarcys* larvae that had been acclimated at 8.5°C were exposed for 15 min at 8.5, 12.5, and 21°C (Fig. 4), and hemolymph was extracted as described previously.

Electron microscopy

A single gill tuft was dissected at the base from a *P. californica* larva and fixed in 75% ethanol/25% water for 24 h. The gills were then held through two changes of 100% ethanol for 2 h each. The sample was critically point dried from carbon dioxide dryer following the method of Anderson [25]. The dried specimen was mounted on an aluminum planchette with DUCO cement (Devcon, Wood Dale, IL, USA). The mounted specimen was coated with approximately 10 nm of 60/40 weight % Au/Pd using an Edwards S150B sputter coater (BOC Edwards, Wilmington, MA, USA) operating at 1×10^{-2} Torr, 5-mbar Argon pressure, 1.5 kV, 20-mA plasma current for 60 s. Examinations were made using the AmRay 3300FE scanning electron micrograph (Bedford, MA, USA) in the Electron Microscope Facility, Department of Botany and Plant Pathology, Oregon State University (OR, USA). Images were recorded on Polaroid (Cambridge, MA, USA) Type 55 P/N positive/negative 4 × 5-inch format film (Fig. 1A and B).

Dual label ^3H_2O and ^{14}C-chlorpyrifos uptake

The ^{14}C-labeled chlorpyrifos was obtained from Dow chemical (Midland, MI, USA). In this study, ^{14}C-chlorpyrifos and ^3H_2O uptake rates were simultaneously compared under two thermal regimes in both the mayfly *Cinygma* sp. and hemipteran *S. washingtonensis*. Artificial water (Fisher 0.67 mM $CaCl_2$, 0.3 mM $MgSO_4$, 1.2 mM $NaHCO_3$, and 0.5 mM KH_2PO_4) was used for all experiments. Fifty milliliters of artificial water, 20 µl of [^3H]-H_2O, and 20 µl of [^{14}C]-chlorpyrifos (in 50:50 acetone:water) were added to Erlenmeyer flasks. This yielded final specific activities for [^3H]-H_2O and [^{14}C]-chlorpyrifos of approximately 6.77 and 0.023 µCi/L, respectively. This [^{14}C]-chlorpyrifos activity corresponds to a concentration of 240 ng/L.

All insects were acclimated to 8.5°C for at least 5 d prior to experimentation. Assays were run at both acclimation temperature and 13°C for both taxa. One individual insect was placed in each flask. Individuals were held for 0, 1, 2, 4, or 8 h. Five insects per time point were used. Following exposure, insects were removed from exposure flasks, rinsed profusely with water, weighed, and digested in Amersham NCS II® (Piscataway, NJ, USA) tissue solubilizer in large scintillation cocktail vials. The digests were held at 50°C overnight and neutralized with glacial acetic acid to obtain pH 7. Eighteen milliliters of Amersham BCS-NA® nonaqueous scintillation cocktail were added, and samples were held in a refrigerator in the dark for at least 4 d to minimize chemiluminescence. These samples were mixed well and counted with a Beckman LS 6500 liquid scintillation counter. Five control insects of each taxa were digested as described previously. The average of control ^{14}C and ^3H activities were taken to be background for each species tested and subtracted from the counts of subsequent time points.

Statistical analysis

Nonlinear regression models were used for initial *P. californica* water influx studies and dual label experiments with *S. washingtonensis* and *Cinygma* sp. Linear regression models, unpaired *t* tests, and analysis of variance were used for instantaneous water influx studies in *P. californica*.

RESULTS

^3H_2O flux in Pteronarcys californica

Acute temperature elevation increased the water uptake rate relative to uptake at acclimation temperature at each time point tested (Figs. 2 and 3). For organisms acclimated at 8.5°C, accumulation of ^3H_2O was characterized by an initial linear response that decayed over time (Fig. 2). One-phase exponential association models were used to described these data with the form $Y = Y_{max}(1 - e^{-kx})$, with k values of 0.032 ± 0.0009 at 8.5°C and 0.08 ± 0.01 when the temperature is shifted to 19.0°C. The r^2 values for these curves are 0.87 and 0.86, respectively. In order to obtain initial rates of water influx, subsequent studies focused on water uptake within relatively short ^3H_2O exposure durations.

Initial water influx rates for *Pteronarcys* are shown in Figure 3. Linear regression models were fit to all instantaneous water influx date. Animals acclimated and exposed to ^3H_2O at 8.5°C had water influx rates of 1.78 ± 0.18 µl/g/min. Animals acclimated and exposed to ^3H_2O at 13°C had water influx rates of 1.75 ± 0.20 µl/g/min. These rates were not statistically different (unpaired t test, $p = 0.85$) and suggest that within a

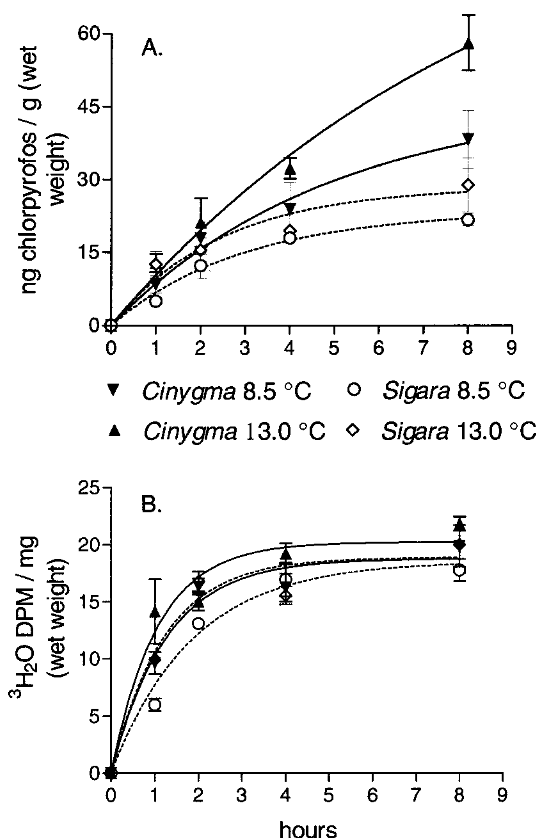

Fig. 6. At acclimation temperature (8.5°C), chlorpyrifos uptake rates were uniformly higher in *Cinygma* sp. than in *Sigara washingtonensis*. Temperature-induced increase in chlorpyrifos uptake was substantially higher in *Cinygma* than in *Sigara* (**A**). At acclimation temperature (8.5°C), water uptake rates (measured as disintegrations per minute [dpm]) were uniformly higher in *Cinygma* than in *Sigara*. Temperature-induced increase in water uptake was similar in *Cinygma* and *Sigara* (**B**). Error bars represent standard errors.

particular temperature range, acclimated *P. californica* maintained a constant permeability. When animals acclimated to 8.5°C were shifted to 13°C, water influx rates increased by 45% to 2.57 ± 0.27 μl/g/min. When animals acclimated to 8.5°C were shifted to 19°C water influx rates increased 3.6-fold to 6.41 ± 1.24 μl/g/min (one-way analysis of variance, $p < 0.01$).

In any given experiment, body weight was not a statistically significant explanatory variable. However, when a multiple linear regression model was fit for all experiments in which *P. californica* were acclimated to 8.5°C, body weight was statistically significant ($p < 0.01$) and negatively related to ^3H_2O activity in the hemolymph.

Comparisons among taxa

Large differences were observed in the intrinsic water permeability of air-breathing and gill-bearing taxa (Figs. 5 and 6). For example, no significant accumulation of ^3H_2O occurred into hemolymph of the air breather *N. kirvyi* that had been acclimated and exposed to ^3H_2O at 8.5°C for up to 9 min (data not shown). This contrasted significantly with *P. californica*, which, under identical acclimation and exposure regimes, accumulated hemolymph ^3H_2O activities that approached 50 dpm/μl (Fig. 2). Considering that *N. kirvyi* weighed, on average, one-ninth of *P. californica*, the relative amounts of water influx required to obtain measurable concentrations in the hemolymph were considerably smaller.

Temperature shifts increased water accumulation in all taxa tested. When three *N. kirvyi* individuals acclimated at 8.5°C were shifted to 13°C for 15 min, the average hemolymph ^3H_2O activity was 18.8 ± 2.3 dpm/μl. A shift from 8.5 to 21°C significantly increased hemolymph ^3H_2O activity to 47.8 ± 2.7 dpm/μl (Fig. 5). Because of the limited availability of *N. kirvyi*, we were unable to measure hemolymph volumes in this species, making absolute comparisons between these two species difficult, especially since they are so markedly different in size. However, it is evident that they are significantly less permeable than *P. californica*. To reduce the confounding factor of size differences among taxa, experiments were performed with *D. gilvipes* larvae that were only slightly larger than *N. kirvyi*. Insects that averaged 0.14 g were acclimated to 8.5°C and exposed to ^3H_2O at acclimation temperature or 13°C for 15 min. The accumulation of ^3H_2O in the hemolymph was significantly higher than both *N. kirvyi* and *P. californica* (Fig. 5), suggesting that external respiratory surfaces are significantly more permeable to water than other integument. The magnitudes of water influx increases associated with temperature shifts were similar across taxa regardless of respiratory strategy.

Similar patterns were found in the dual label experiments, where the gill-breathing ephemeropteran *Cinygma* sp. was more water permeable than the air-breathing hemipteran *S. washingtonensis* (Fig. 6B). An acute temperature shift increased the magnitude of water influx rates similarly in both taxa. One-phase exponential association curves were fit for these data with the form $Y = Y_{max}(1 - e^{-kx})$. The r^2 values for *Cinygma* sp. uptake curves at 8.5 and 13.0°C were 0.90 and 0.84, respectively. The r^2 values for *S. washingtonensis* uptake curves at 8.5 and 13.0°C were 0.93 and 0.85, respectively.

^{14}C-chlorpyrifos uptake

As was the case for water accumulation, ^{14}C-chlorpyrifos uptake was consistently higher in the gill-bearing mayfly *Cinygma* sp. than in the air-breathing hemipteran *S. washingtonensis* in all experiments (Fig. 6A). One-phase exponential association curves were fit for these data with the form $Y = Y_{max}(1 - e^{-kx})$. The r^2 values for *Cinygma* sp. uptake curves at 8.5 and 13.0°C were 0.88 and 0.69, respectively. The r^2 values for *S. washingtonensis* uptake curves were 0.74 and 0.87, respectively. An acute temperature increase of 4.5°C increased uptake rates in both organisms tested. However, the increases were substantially greater in *Cinygma* sp. After 8 h of exposure, the temperature induced increase in chlorpyrifos uptake was 2.75-fold higher in *Cinygma* sp. than in *S. washingtonensis*.

DISCUSSION

The water permeability of aquatic insects has received little attention recently, as the majority of studies conducted in this area occurred in the 1950s and 1960s [26–29]. We could not find direct comparisons of water influx between air-breathing and water-breathing species until Frisbee and Dunson [9] compared water influx in an air-breathing beetle *Dytiscus verticalis* to water influx in the rectal gill-bearing odonate *Anax junius*. They found not only that the gill breathers were twice as permeable to water but also that they lost sodium to the surrounding water under low pH conditions. The air-breathing *Dytiscus* was relatively impermeable to water (influx) and so-

dium (efflux) and tolerant to low pH. Our interest in exploring the water permeability of freshwater insects stems from the notion that osmotic gradients require that these animals maintain relatively impermeable integuments with the notable exception of gas and solute exchange epithelial surfaces. As these functionally exposed cellular surfaces can be important uptake sites for some contaminants, water permeability differences among taxa may possibly predict uptake potential for many contaminants. Recent work with ten aquatic insect taxa showed that dissolved oxygen-breathing insects with larger exchange epithelial surfaces have significantly higher water uptake rates than air-breathing taxa with smaller exchange epithelial surfaces [17].

Water influx rates in aquatic insects are dependent on a number of factors including respiratory strategy, temperature, acclimation history, and body size. Based on our experiments with *Pteronarcys*, we suggest that focusing on early time points results in more accurate water influx rate estimates for a number of reasons. Water efflux rates become significant when the blood or hemolymph ^3H_2O concentrations approach 4 to 7% of the external medium (L.B. Kirschner, Washington State University, Pullman, WA, USA, personal communication). Therefore, hemolymph samples taken beyond the initial linear portion of the time-^3H_2O accumulation curves (Fig. 2) would result in underestimating influx rates. Because ^3H_2O distributes to total body water via diffusion from the hemolymph, analysis of hemolymph is advantageous for instantaneous uptake rates. However, whole-body accumulation of ^3H_2O is less labor intensive and allows for direct comparison of permeability differences among taxa.

Acute temperature changes but not acclimation temperatures were important factors in water influx rates in *P. californica* (Fig. 4). Insects that were acclimated and exposed to ^3H_2O at 8.5 and 13°C, respectively, did not differ with respect to influx rates. This suggests that *P. californica* effectively acclimated to these temperatures. The 45% increase in water influx rate observed when organisms were shifted from 8.5 to 13°C may be due to increased metabolic and circulatory rates, increased fluidity of the gill plasma membrane, and/or decreased tightness of cell-to-cell junctions in epithelial surfaces. We observed a 3.6-fold increase in water influx rate when larvae were subjected to an acute temperature shift to 19°C under these conditions. While a 10.5°C acute temperature increase is not environmentally realistic, we speculate that the observed flux increase is due to more than simply an increase in metabolic rate. We did not observe the characteristic push-up ventilatory behavior in *Pteronarcys* until nearly 5 min of exposure to this extreme temperature increase and can rule out increased ventilation rates as a possible explanatory factor. We speculate that extreme temperature changes cause physical changes in the gill epithelium, resulting in increased permeability.

Insect species that breathe through external epithelia and gills are significantly more water permeable than air-breathing insects (Figs. 5 and 6A). Furthermore, we demonstrate that temperature increases result in increased water influx rates. Sufficient temperature elevations result in increased metabolic rate in both air breathers and water breathers. However, water breathers are at a distinct disadvantage in these situations because oxygen availability decreases and water fluxes increase substantially. Based on the metabolic costs associated with removing excess water and maintaining homeostatic processes, we suggest that insects with large epithelial surfaces may be more susceptible to temperature changes. Increased ^3H_2O uptake was presumably related to temperature-induced changes in the physical state (cell membrane fluidity and/or cell-to-cell tight junctions) of the respiratory epithelium as well as increases in metabolic rates. Figure 1 shows the extensive gill surface area of *P. californica* (Fig. 1A) as well as the chloride cell–rich epithelial tissue at the base of each gill tuft (Fig. 1B). Chloride cells are presumably necessary to acquire necessary cations from the water column and compensate for diffusive ion losses.

Accumulation of ^{14}C-chlorpyrifos was uniformly greater in the mayfly *Cinygma* sp. than in the hemipteran *S. washingtonensis* on a per-weight basis (Fig. 6A). This is notable because *Cinygma* was on average 45% heavier than *Sigara*. These results agree with previous findings that dissolved oxygen-breathing organisms generally have higher ^{14}C-chlorpyrifos uptake rates than air-breathing taxa [17]. As expected, an acute temperature increase of 4.5°C increased ^{14}C-chlorpyrifos accumulation rates in both the ephemeropteran *Cinygma* sp. and in the hemipteran *S. washingtonensis*. However, the increases were more pronounced in the gill bearing *Cinygma* than in the air-breathing *Sigara* (Fig. 6). This suggests that epithelial surfaces play a key role in determining uptake of chlorpyrifos and that organisms with larger epithelial exchange surface areas are potentially more susceptible to temperature increases in contaminated environments.

We suggest that differences in respiratory and osmoregulatory strategies may help explain the marked differences in sensitivities to water quality problems that have been observed in insect species. In polluted environments, organisms with relatively large exchange epithelial surfaces are likely more sensitive to a number of environmental stressors (temperature, dissolved oxygen, pH changes, and contaminant exposure) that interact via different mechanisms through these surfaces.

It is likely that many generalizations regarding taxa sensitivities/tolerances that provide the basis for many biotic indices for assessing water quality have physiological underpinnings. All Ephemeroptera, Plecoptera, and Trichoptera taxa have closed respiratory systems, and many have extensive respiratory and osmoregulatory cell surface areas on the integument. Gill surfaces [30] and anal papillae [31] can be damaged by environmental stressors such as chlorinated compounds, crude oil wastes, and heavy metals, respectively. Many taxa generalized to be relatively tolerant to water chemistry–associated stressors lack the characteristic of having extensive epithelial surfaces. For example, the salinity tolerance of the mosquito *Toxorhynchites haemorrhoidalis* (Fabricus) relative to other mosquito species may be a consequence of its low cuticular permeability [10]. Similarly, heptageniid mayflies, which typically have extensive gill surface areas, tend to be more sensitive to metal pollution than baetid mayflies, which tend to have significantly smaller gills [32].

Understanding the mechanistic bases for differences in species sensitivities to individual and multiple stressors is an essential step in assessing water quality impacts in nature. Further experimental work that helps elucidate important organismal characteristics that determine species' responses to environmental stressors can potentially be useful in refining current bioassessment techniques. Within a taxonomic framework, it may be possible to establish stressor-specific tolerance values based on defensible biological characteristics that are known to be important determinants of species' responses to specific stressors. This would be a vast improvement over the

current use of single tolerance values for species across all stressor types.

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