Eurasia Specialized Veterinary Publication

Entomological Research Letters

ISSN: 3108-639X

2024, Volume 4, Issue 2, Page No: 73-83
Copyright CC BY-NC-SA 4.0
Available online at: www.esvpub.com/

Ion Transport Constrains Whole-Animal Water Balance, Salinity Tolerance, and Thermal Performance Across Insect Environments

Emily Johnson[1]*, Robert Smith[1], Laura Brown[2], Kevin Miller[3]

[1]Department of Ion Transport and Water Balance, Faculty of Life Sciences, University of Toronto, Toronto, Canada.
[2]Department of Salinity Tolerance and Thermal Performance, Faculty of Biosciences, University of British Columbia, Vancouver, Canada.
[3]Department of Environmental Physiology and Ion Regulation, Faculty of Sciences, McGill University, Montreal, Canada.

***E-mail** ✉ emily.johnson@utoronto.ca

ABSTRACT

Ion transport is commonly treated as a set of tissue-specific processes controlling excretion, osmoregulation, and ionic balance. That framing becomes incomplete when the biological question concerns whole-animal performance across contrasting insect environments. Epithelial systems that generate, recover, or redirect ionic gradients also influence osmotic water movement, extracellular ion stability, membrane function, and responses to hydric, saline, and thermal stress. Yet increased transport is not inherently beneficial, transport capacity cannot be inferred from molecular abundance alone, and organismal tolerance can improve even when conventional ion-regulatory metrics do not. This article develops a proposed physiological constraint framework in which environmental ionic demand is evaluated against epithelial transport capacity, regulatory state, compartmental selectivity, water permeability, energetic buffering, and non-renal co-limiters. The framework distinguishes compensated challenge, transport-associated constraint, and performance that becomes partly decoupled from measured transport because other mechanisms dominate or compensate. Evidence across mosquitoes, Drosophila, mayflies, locusts, lepidopteran larvae, and cold-specialized beetles supports individual relationships within this architecture but not a universal causal hierarchy. Ion transport is therefore treated as a context-dependent constraint on hydration, salinity tolerance, and thermal performance rather than as a single master determinant.

Keywords: Malpighian tubules, Hindgut ion transport, Osmoregulation, Salinity stress, Thermal physiology, Insect water balance

Received: 11 September 2024
Revised: 01 November 2024
Accepted: 02 November 2024

How to Cite This Article: Johnson E, Smith R, Brown L, Miller K. Ion Transport Constrains Whole-Animal Water Balance, Salinity Tolerance, and Thermal Performance Across Insect Environments. Entomol Res Lett. 2024;4(2):73-83.
https://doi.org/10.51847/bgwHUWcWbC

Introduction

Water balance in insects emerges from coordinated gains, losses, storage, and recovery rather than from a single renal process. Malpighian tubules and the hindgut contribute through ion recycling and epithelial water recovery, but cuticular permeability, respiratory loss, drinking, feeding, and behavior can alter the same whole-animal outcome [1]. Consequently, strong epithelial water conservation need not imply low total water loss, and hydration maintained behaviorally need not require maximal renal retention.

Hydric state also changes how thermal performance should be interpreted. During sufficiently prolonged hot and dry exposure, dehydration may become increasingly important to performance loss or mortality, whereas direct temperature-dependent cellular injury may dominate under other conditions [2]. At low temperature, disruption

© 2024 Entomological Research Letters

of ion and water homeostasis is central to chill injury in many susceptible insects, but membranes, metabolism, repair processes, and other physiological systems also contribute [3]. The analytical problem is therefore not whether ion transport matters, but when its state becomes limiting relative to environmental demand and competing physiological constraints.

Salinity illustrates the same issue. Together, studies in *Neocloeon triangulifer* show that responses to low-ion and major-ion environments are physiologically plastic and depend on ionic context [4, 5]. Such evidence argues against treating "salinity" as one physiological variable. Ionic composition, concentration, exposure history, tissue specialization, and compensatory capacity can all change the consequences of altered transport.

This article develops a proposed physiological constraint framework spanning epithelial ion movement, Malpighian and hindgut function, osmotic water movement, salinity challenge, temperature dependence, energetic consequences, and organismal performance. It does not assume that higher transport is beneficial or that a single biomarker diagnoses limitation. Instead, it asks whether environmental ion and water demands can be met by the insect's state-dependent capacity to generate, redirect, conserve, or eliminate ions and associated water, and whether other systems amplify, buffer, or bypass that relationship.

Epithelial ion transport

Insect ion transport is generated by combinations of pumps, cotransporters, channels, exchangers, paracellular pathways, and endocrine control. A central energizing component in many insect epithelia is the V-type H+-ATPase, but its activity is not fixed. In *Aedes aegypti* Malpighian tubules, CAPA antidiuretic signaling inhibits diuretic-stimulated V-ATPase through a nitric oxide–cGMP–protein kinase G-associated pathway involving reversible V1/Vo dissociation [6]. This establishes direct regulation of a major transport driver in a mosquito renal epithelium, not a universal endocrine mechanism for insects.

Transporter identity and localization further define epithelial capacity. Functional characterization of mosquito aeCCC2 and the *Drosophila* Ncc83 ortholog demonstrates electrogenic cation–chloride transport properties, while aeCCC2 localization identifies a basolateral route potentially contributing to mosquito Malpighian ion movement [7]. Such functional evidence is stronger than expression alone, although heterologous transport measurements cannot specify the transporter's quantitative in-vivo contribution under every state. Larval *A. aegypti* Malpighian tubules and anal papillae also express voltage-gated channel candidates, with selected perturbations altering epithelial electrical properties during salt and water imbalance [8]. Channel expression and electrical effects therefore identify regulatory capacity without proving whole-animal salinity tolerance.

Transport is heterogeneous even within one renal organ. In larval *Trichoplusia ni*, chloride handling varies among Malpighian-tubule regions and cell types and can change with physiological regulation [9]. In the same species, Na+ and K+ handling within the distal ileac plexus can switch between secretion and reabsorption according to ion-loading state, with regional ion recycling supported by gap-junctional organization [10]. A single value for "renal secretion" can therefore conceal biologically important differences in direction, selectivity, and compartmental function.

A second boundary concerns evidence class. Transcriptomic profiles can reveal candidate transport machinery, but functional interpretation is strongest when expression evidence is integrated with direct physiological measurements [11]. Even direct elevation of transport cannot automatically be interpreted as organismal benefit. Tachykinin signaling increases fluid and ion transport by adult *Drosophila melanogaster* Malpighian tubules [12], yet enhanced diuresis could have different consequences after feeding, during water abundance, or during dehydration. The proposed framework therefore separates molecular availability, epithelial flux, regulatory state, and organismal consequence.

Malpighian tubules and hindgut

The Malpighian tubules and hindgut constitute a coupled but anatomically diverse system in which secretion, reabsorption, timing, and selectivity can each become limiting. In *Rhodnius prolixus*, downregulation of the glycoprotein hormone receptor LGR1 changes Malpighian-tubule secretion and the timing of post-prandial diuresis [13]. This connects endocrine state to rapid post-feeding water handling in a blood-feeding hemipteran, while its specialized feeding biology limits extrapolation to other insects.

Hindgut epithelia may also be configured for active salt elimination. In larvae of the saline-water mosquito *Aedes taeniorhynchus*, posterior rectal Na+ secretion is consistent with a proton-driven system involving V-H+-ATPase-supported Na+/H+ exchange [14]. This differs from the ion-conserving roles emphasized for many freshwater and

terrestrial systems. The transferable principle is therefore functional flexibility of hindgut transport, not conservation of one molecular topology across taxa.

Temperature exposes another dimension: selective transport can matter more than total flux. Cold-tolerant or cold-acclimated *Drosophila* states maintain more favorable hindgut fluid and Na+ reabsorption while limiting maladaptive K+ reabsorption at low temperature [15]. In *D. melanogaster*, cold acclimation similarly improves Malpighian and rectal ion-handling function and mitigates hyperkalemia even though measured ATPase activities are lower or unchanged [16]. Bulk transporter-enzyme activity is consequently an incomplete proxy for integrated epithelial performance. Within the proposed framework, Malpighian and hindgut capacity is therefore compartmental, selective, temporally regulated, and environmentally conditional.

Osmotic water movement

Ion transport affects hydration because solute movement establishes gradients along which water can move through transcellular and paracellular routes. The water consequence of an ion flux nevertheless depends on epithelial permeability, transport direction, endocrine state, and whether water should be retained or eliminated in the prevailing physiological context.

Ion transport peptide illustrates this cross-level coordination in *Drosophila*. Manipulation of ITP signaling alters drinking, excretion, and dehydration-related phenotypes, demonstrating that water intake and excretory control are coordinated neuroendocrinely [17]. Because ITP has effects beyond a single renal tissue, these experiments do not justify attributing whole-animal water balance exclusively to Malpighian transport.

Water-handling machinery also changes with physiological state. In female *A. aegypti*, Malpighian-tubule phosphoproteomes differ markedly between unfed and post-blood-meal conditions, including changes involving transport-associated proteins [18]. These molecular transitions are compatible with rapid reconfiguration during a major osmotic and volume load, but phosphorylation does not itself establish net water flux.

Aquaporin evidence adds a direct permeability component. In *Anopheles sinensis*, aquaporin isoforms differ in water and solute permeability, occur in osmoregulatory tissues, and display feeding- and temperature-responsive expression [19]. The results demonstrate that water-channel identity can modify epithelial permeability while leaving the organism-level importance of individual isoforms context dependent.

Because ion-gradient production, endocrine coordination, and water permeability can vary independently, a side-by-side classification is required to prevent mechanistically different observations from being interpreted as the same osmotic response.

Table 1. Osmotic water-movement states that determine what epithelial ion transport can legitimately imply for hydration

Position in the water-balance chain	Context and transport evidence	Physiological implication and limiting link	Paired measurement requirement and state
Gradient production and post-load reconfiguration			
Gradient generation with a permissive water pathway	Renal or hindgut epithelium Direct ion movement accompanied by an established water route	Ionic transport can support directional osmotic water movement Water permeability or paracellular conductance may remain limiting	Measure solute and water flux in the same epithelial state Evidence-supported principle
Post-load renal reconfiguration	Large feeding-associated osmotic or volume load State-dependent phosphorylation of transport-associated machinery	Water-handling capacity is molecularly dynamic Molecular change does not establish direction or magnitude of water flux	Pair molecular profiling with functional secretion or permeability assays Evidence-supported boundary
Transcellular permeability and discordance			
Isoform-dependent transcellular permeability	Aquaporin-expressing osmoregulatory tissue Direct differences in channel permeability	Equivalent ionic gradients can produce different water consequences Heterologous channel function may not equal native contribution	Resolve isoform-specific function in native epithelia Evidence-supported mechanism

Gradient–permeability discordance	Ion transport and water permeability vary independently Divergence between ion-transport and hydration-related endpoints	Strong ion transport need not yield proportionate water movement The mismatch has not been defined by a universal threshold	Measure both components before inferring hydration benefit **Proposed synthesis**
Whole-animal intake-excretion coordination			
Coordinated intake–excretion state	Neuroendocrinely regulated whole animal Manipulated signaling changes drinking and excretion	Hydration reflects coordinated water acquisition and elimination Behavioral and metabolic effects may accompany epithelial regulation	Separate intake, excretion, and epithelial transport endpoints Evidence-supported principle

Salinity challenges

Salinity challenge is mechanistically heterogeneous because different dissolved ions impose different acquisition, elimination, toxicity, and compensatory demands. Under dilute Na+ conditions, *N. triangulifer* can increase sodium-uptake capacity while maintaining measured growth, survival, and development across the tested experimental range [4]. In contrast, exposure to different major-ion salts produces ion-specific acclimatory and pathological responses, including distinct effects associated with elevated Ca2+ [5]. Environmental ionic demand therefore cannot be represented adequately by conductivity or total salinity when the mechanism under consideration is ion specific.

Molecular remodeling is likewise composition dependent. Gill proteomics in *N. triangulifer* reveals different responses to different major-ion environments, including changes in recognized ion-transport machinery as well as expected candidates that were undetected or unchanged [20]. These results demonstrate tissue-level molecular responsiveness but do not establish the direction or magnitude of whole-animal ion flux. Protein abundance, functional transport, and organismal tolerance remain separate evidential levels.

Mosquito studies show an additional distinction between generalized plasticity and specialized epithelial solutions. Larval *A. aegypti* changes channel-related properties in ionoregulatory tissues between freshwater and brackish conditions [8]. The saline-water specialist *A. taeniorhynchus*, however, possesses posterior rectal machinery capable of active Na+ secretion [14]. These observations should not be interpreted as one conserved pathway or an evolutionary sequence. They demonstrate that the tissue location, direction, and molecular basis of salt handling can differ substantially among environmental contexts.

In the proposed framework, salinity becomes transport-constraining when environmental ionic demand cannot be compensated without destabilizing internal ion concentrations, epithelial function, or associated water distribution. No universal threshold is supported. Demonstrating such a constraint requires matched environmental chemistry, epithelial physiology, internal ion status, and organismal performance rather than inference from salinity exposure alone.

Temperature dependence of transport

Temperature can alter transport kinetics and selectivity while simultaneously changing membranes, metabolism, and other cellular processes. Across five *Drosophila* species, cold tolerance is strongly associated with the capacity of Malpighian tubules and rectal pads to maintain favorable K+ transport at low temperature [21]. This provides important comparative evidence for a transport constraint within that taxonomic system, but covariance does not establish epithelial K+ handling as the sole cause of interspecific cold-tolerance differences.

Experimental and acclimatory manipulations likewise show that ionic state and its regulation can alter chill performance [22, 23]. Cold acclimation in *Locusta migratoria* preserves extracellular ion balance and muscle membrane potential alongside improved chill tolerance [22]. In *Drosophila*, CAPA manipulation changes renal K+ handling and chill tolerance in a dose-dependent manner, with high-dose effects differing from effects at lower concentrations [23]. The common inference is that ionic regulation matters to chill performance; the endocrine and organ-level mechanisms should not be treated as equivalent.

Exposure duration and thermal strategy further modify the relationship. Larval *Dendroctonus ponderosae* can survive prolonged stressful cold while mitigating systemic ionoregulatory collapse [24]. This cold-specialized beetle differs from the chill-susceptible dipteran models that dominate mechanistic work, making taxonomic strategy and duration important boundaries on generalization.

Cold performance is also not reducible to extracellular K+ regulation. Across *Drosophila* species, low-temperature mitochondrial performance covaries with cold tolerance and provides a credible cellular co-limiter [25]. Fluctuating thermal regimes can mitigate chill injury through interacting ion-homeostatic, membrane, oxidative, metabolic, and repair processes rather than a single epithelial mechanism [26]. Most critically, dietary K+ and cold acclimation can improve *D. melanogaster* cold tolerance even without improved extracellular K+ handling, because changes in intracellular K+ can help preserve transmembrane gradients [27]. An extracellular ion concentration, therefore, is not interchangeable with epithelial transport capacity, intracellular ionic state, membrane potential, or organismal performance.

The interpretive problem is best exposed by registering the thermal contexts that produce concordance or discordance among these variables rather than treating every cold-associated ion change as the same physiological event.

Table 2. Low-temperature transport states that distinguish epithelial constraint from ionic compensation and non-renal co-limitation

Temperature-dependent state	System examined and ionic pattern	Physiological consequence	Co-limiter or rival mechanism	Discriminator with evidence position
Epithelial and systemic preservation				
Preserved epithelial K+ selectivity	Comparative *Drosophila* renal and hindgut systems Favorable K+ secretion or reabsorption retained during cold	Consistent with reduced ionoregulatory disruption and better chill tolerance	Comparative covariance does not isolate sole causality	Manipulate epithelial transport while controlling other thermal traits Evidence-supported association
Preserved systemic ions with maintained excitability	Cold-acclimated locust Extracellular ionic stability accompanies preserved membrane potential	Connects systemic ion balance with cellular and organismal function	Organ source of preserved ionic state is incompletely isolated	Combine organ-specific flux, haemolymph ions, and electrophysiology Evidence-supported relationship
Endocrine and prolonged compensation				
Endocrine transport nonlinearity	CAPA-manipulated *Drosophila* Renal ion handling and chill outcome depend on peptide dose	Regulatory state can alter both magnitude and direction of outcome	Supra-physiological exposure may not represent endogenous signaling	Resolve endogenous concentrations and dose-specific renal responses Evidence-supported nonlinearity
Prolonged mitigation of ionoregulatory collapse	Cold-specialized beetle larva Systemic ionic disruption is limited during extended cold	Ionoregulatory failure can be delayed rather than immediate	Cold-specialist physiology may not resemble chill-susceptible models	Compare transport kinetics across thermal strategies and durations Evidence-supported boundary case
Mitochondrial or extracellular-ion decoupling				
Mitochondrial co-limitation	Comparative *Drosophila* physiology Low-temperature respiratory impairment varies with species tolerance	Adequate epithelial regulation may not remove another cellular constraint	Mitochondrial and ionoregulatory traits can covary	Measure both systems under matched thermal conditions Evidence-supported co-limiter
Extracellular-K decoupling	Dietary-K and acclimation manipulation Cold tolerance improves without better extracellular-K regulation	A conventional extracellular metric can miss compensatory ionic states	Intracellular ion pools alter transmembrane-gradient stability	Measure intra- and extracellular ions with membrane potential Evidence-supported counterexample
Capacity-margin synthesis				
Cold transport-capacity margin	Cross-system interpretation Multiple transport and ionic-state variables considered jointly	Constraint is expected only when compensatory	No universal threshold or validated cross-	Test multilevel predictions prospectively across taxa and environments

	regulation becomes insufficient	taxon index exists	**Proposed synthesis**

Energetic costs

Active ion transport requires energy, but the energetic consequence of osmoregulation cannot be inferred directly from transporter activity or epithelial flux. In a comparative study of aquatic invertebrates, freshwater insect species increased ion-transport activity across salinity conditions without a detectable increase in whole-animal metabolic rate [28]. This does not mean that epithelial transport is energetically free. Tissue-specific ATP expenditure may be offset by reduced expenditure elsewhere, absorbed within resting metabolic variation, or too small to resolve with whole-animal respirometry. It does show that transport demand and organismal metabolic rate are not interchangeable measurements.

Endocrine coupling adds another level. Manipulation of ion transport peptide in *Drosophila* changes feeding, metabolic expenditure, and energy stores [29]. These effects connect water- and ion-regulatory signaling to systemic energy balance, but they do not quantify the ATP cost of Malpighian pumping. Likewise, cold-acclimated *Drosophila* can improve organ-level ion handling without higher measured ATPase activity [16]. Energetic interpretation therefore requires level-matched evidence: transporter biochemistry, epithelial flux, tissue ATP use, and whole-animal metabolism should remain analytically separate.

The proposed framework consequently treats energetic cost as a conditional modifier of transport capacity rather than a fixed surcharge on every unit of ion movement. Thermal history can also alter repair, membrane, oxidative, and metabolic demands [26], meaning that the same measured epithelial flux could occur under different energetic constraints.

A useful consequence of this separation is that energetic limitation should be diagnosed by discordance, not assumed from transport intensity. If epithelial flux rises while whole-animal metabolic rate is unchanged, the next question is whether local ATP demand has been compensated, whether another process has reduced its expenditure, or whether the measurement lacks tissue-level resolution. Conversely, a rise in whole-animal metabolism during osmotic stress need not identify epithelial pumping as its source because locomotion, stress signaling, repair, ventilation, and biosynthesis can contribute. The proposed model therefore treats an energetic constraint as supported only when the energetic measurement is matched to the biological level at which transport demand is being interpreted.

This discordance is sufficiently important to require visual separation of evidence-supported measurements from proposed inferences. **Figure 1** is positioned here because energetic claims become misleading when molecular activity, epithelial work, systemic metabolism, and performance are collapsed into one axis; the figure triages these discordant evidence levels before the framework proceeds to whole-animal outcomes.

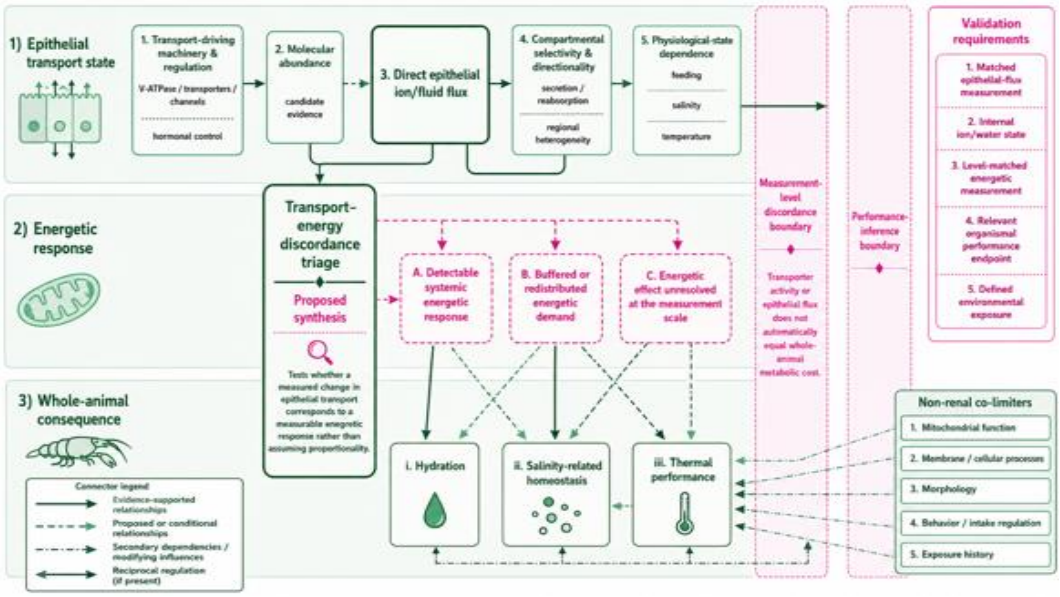


Figure 1. When transport demand, energetic expenditure, and organismal performance cease to move together

Whole-animal hydration and performance

Performance is the point at which transport-centered explanations face their strongest test. Dietary salt supplementation in *Drosophila* alters body ion status and modifies thermal-acclimation responses of flight performance, while morphological traits also change [30]. Flight is therefore an organismal endpoint influenced by ionic state but is not a specific readout of renal transport. Similar caution applies to survival, chill-coma recovery, growth, or development: each integrates multiple physiological systems.

Compensation further complicates inference. Mayfly nymphs can alter Na+ uptake while maintaining measured developmental and survival endpoints under tested dilute conditions [4]. Cold acclimation can improve renal and rectal transport alongside improved chill tolerance [16], whereas increased dietary K+ can improve cold tolerance without improving extracellular K+ regulation [27]. These cases support a distinction between measured transport state and regulatory margin: an animal may preserve performance by changing transport, redistributing ion pools, or recruiting another physiological route before a conventional biomarker indicates failure.

Whole-animal hydration adds external routes that renal measurements cannot capture. Cuticular and respiratory losses, intake, storage, and behavioral water acquisition remain part of total water balance [1]. Under prolonged hot-dry conditions, exposure duration and atmospheric water demand can alter whether dehydration contributes materially to performance decline [2]. The evidence coverage therefore becomes uneven precisely where laboratory organ physiology must be transferred to field-relevant performance.

The following map separates well-supported cross-level relationships from gaps where organismal inference still requires additional measurements rather than another summary of individual studies.

Table 3. Coverage and unresolved transfer gaps between epithelial homeostasis and whole-animal performance

Transfer bridge	Present evidence coverage	Whole-animal claim ceiling	Missing link and boundary condition	Priority validation
Compensated ion challenges				
Compensated dilute-ion challenge	Direct uptake physiology plus measured developmental endpoints	Transport plasticity can preserve measured performance	Energetic or later-life costs may remain unmeasured Freshwater mayfly laboratory conditions	Couple uptake, water balance, energy use, and later performance
Cold-acclimated epithelial compensation	Organ flux, systemic ions, and chill phenotype jointly available	Improved transport can accompany improved tolerance	Contribution relative to other acclimatory changes remains unresolved *Drosophila* laboratory acclimation	Selectively perturb transport while measuring co-limiters
Intracellular-ion compensation	Extra- and intracellular ionic states plus cold performance	Performance may improve without better extracellular-K regulation	Which ionic compartment best predicts failure is context dependent Dietary-K manipulation in *Drosophila*	Measure gradients, membrane potential, and organ flux together
Discordant locomotor or hydration outcomes				
Salt-associated locomotor change	Body ions and flight performance measured with morphology	Ionic environment can influence performance without being performance-specific	Morphological and other mediators prevent renal attribution Adult *Drosophila* diet/acclimation	Partition ionic, morphological, and energetic contributions
Hydration under dry heat	Mechanistic water-balance traits with climate-relevant interpretation	Dehydration may become increasingly limiting with duration and dryness	Cross-taxon field thresholds are not validated Exposure humidity, duration, and behavior	Validate coupled hydric–thermal measurements under realistic exposures
Proposed transport-to-performance constraint				
Transport-to-performance constraint	Cross-level evidence assembled across systems	Performance should deteriorate when compensatory	No universal threshold or prospective cross-taxon validation exists	Test the proposed relationship prospectively using

	transport margin is exhausted and co-limiters cannot buffer disturbance	Taxon, stage, environment, and exposure history	matched multilevel measurements

Integrated constraint model

The central contribution of this article is a proposed constraint model, not an empirically validated universal mechanism. Its starting variable is environmental ionic and hydric demand: the combination of ion composition, water availability, temperature, and exposure history that changes what an epithelium must acquire, conserve, redistribute, or eliminate. The opposing variable is state-dependent transport capacity, defined qualitatively by transporter availability, epithelial directionality, compartmental selectivity, endocrine regulation, and water permeability. Evidence that these properties can change independently argues against reducing capacity to one transporter or secretion rate [6].

A transport-associated constraint is proposed when environmental demand approaches or exceeds the compensatory capacity of relevant epithelia and internal ionic or water states begin to destabilize. Specialized salt-secreting rectal machinery illustrates that the relevant capacity can be anatomically specific [14], whereas cold acclimation demonstrates that capacity can be altered plastically [16]. The model therefore predicts relationships, not fixed thresholds.

Three departures from a simple demand–capacity relationship are essential. First, compensation can occur through altered ion pools, water permeability, behavior, or other tissues. Second, energetic expenditure may be buffered such that increased transport is not visible as higher whole-animal metabolic rate [28]. Third, non-renal co-limiters can dominate; mitochondrial thermal sensitivity [25] and morphology-associated performance variation [30] provide concrete examples. Transport can thus be necessary to preserve homeostasis in a particular context without being sufficient to explain the organismal endpoint.

This distinction also changes how negative findings should be used. Failure to detect a change in extracellular K+, metabolic rate, or one transporter cannot by itself falsify a transport contribution if compensation occurs in another compartment or timescale. Equally, a strong molecular response cannot establish organismal importance unless it changes a physiologically relevant flux or state. The framework is intended to make these asymmetries explicit: positive molecular evidence, negative whole-animal evidence, and preserved performance can coexist without contradiction when they refer to different levels of organization. A rigorous test must therefore specify which level is expected to fail first and which compensatory routes remain available.

Validation should compare the proposed multilevel architecture against simpler explanations. Direct epithelial flux, internal ions, water state, metabolic measurements, membrane function, and performance should be synchronized across defined exposures. Field transfer additionally requires realistic humidity, temperature fluctuation, ionic chemistry, diet, behavior, and life stage. Until such tests exist, the model organizes mechanistic hypotheses and evidence boundaries rather than providing a predictive score.

Results and Discussion

The framework shifts the question from whether ion transport affects insect performance to when transport becomes constraining and when compensation or co-limitation prevents a one-to-one relationship. Taxonomic evidence already shows distinct solutions: saline-water mosquito larvae use specialized rectal salt secretion [14], mayfly gills remodel molecularly in ion-specific environments [20], and mountain pine beetle larvae mitigate ionoregulatory collapse during prolonged cold [24]. These examples justify a general comparative problem, not a universal epithelial topology.

Terminology must also follow study design. Changes induced by dilute media, thermal acclimation, or short-term temperature exposure demonstrate physiological plasticity or state dependence [4]. Comparative preservation of hindgut transport is consistent with a relationship to cold tolerance [15], whereas temperature-responsive aquaporin expression does not alone establish evolved adaptation [19]. Evolutionary claims require evidence beyond molecular response or laboratory acclimation.

The most informative contradictions define the framework's boundaries. Mitochondrial performance can covary with cold tolerance independently of renal measurements [25]. Increased osmoregulatory transport can occur without a detectable whole-animal metabolic-rate rise [28]. Salt-associated flight changes occur alongside

morphology [30]. These findings make co-limitation and cross-level decoupling central features rather than inconvenient exceptions.

Future work should therefore prioritize matched measurements across levels. Functional epithelial physiology should anchor molecular profiling because expression cannot substitute for transport [11]. Hydric and thermal measurements should be combined under exposure durations relevant to natural stress [2]. Comparative tests should deliberately include different orders, life stages, feeding modes, aquatic and terrestrial environments, and both acute and acclimatory timescales. Such designs can determine whether the proposed constraint relationships recur, change sign, or disappear across biological contexts.

Conclusion

Ion transport provides a mechanistically credible bridge between epithelial physiology and whole-animal water balance, salinity tolerance, and thermal performance, but that bridge is conditional. Transport direction, selectivity, endocrine state, water permeability, intracellular ion distribution, energetic buffering, and non-renal co-limiters can each change the mapping from epithelial activity to organismal outcome. The proposed constraint model therefore rejects both renal reductionism and the opposite assumption that organ-level transport is too context dependent to integrate. Its useful claim is narrower: ion transport becomes physiologically constraining when environmental demand exceeds available compensatory margin and the resulting ionic or hydric disturbance is not buffered elsewhere. Testing that proposition requires synchronized, multilevel measurements across taxa, life stages, and realistic environmental exposures.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

References

1. O'Donnell MJ. A perspective on insect water balance. J Exp Biol. 2022;225(7). doi:10.1242/jeb.242358
2. Sinclair BJ, Saruhashi S, Terblanche JS. Integrating water balance mechanisms into predictions of insect responses to climate change. J Exp Biol. 2024;227(10). doi:10.1242/jeb.247167
3. Overgaard J, MacMillan HA. The integrative physiology of insect chill tolerance. Annu Rev Physiol. 2017;79:187-208. doi:10.1146/annurev-physiol-022516-034142
4. Cochran JK, Buchwalter DB. The acclimatory response of the mayfly Neocloeon triangulifer to dilute conditions is linked to the plasticity of sodium transport. Proc Biol Sci. 2022;289(1979):20220529. doi:10.1098/rspb.2022.0529
5. Orr SE, Watanabe TTN, Buchwalter DB. Physiological plasticity and acclimatory responses to salinity stress are ion-specific in the mayfly, Neocloeon triangulifer. Environ Pollut. 2021;286:117221. doi:10.1016/j.envpol.2021.117221
6. Sajadi F, Vergara-Martínez MF, Paluzzi JPV. The V-type H+-ATPase is targeted in antidiuretic hormone control of the Malpighian 'renal' tubules. Proc Natl Acad Sci U S A. 2023;120(51). doi:10.1073/pnas.2308602120
7. Duong PC, McCabe TC, Riley GF, Holmes HL, Piermarini PM, Romero MF, et al. Sequence analysis and function of mosquito aeCCC2 and Drosophila Ncc83 orthologs. Insect Biochem Mol Biol. 2022;143:103729. doi:10.1016/j.ibmb.2022.103729
8. Farrell S, Dates J, Ramirez N, Hausknecht-Buss H, Kolosov D. Voltage-gated ion channels are expressed in the Malpighian tubules and anal papillae of the yellow fever mosquito (Aedes aegypti), and may regulate ion transport during salt and water imbalance. J Exp Biol. 2024;227(3). doi:10.1242/jeb.246486
9. Kolosov D, O'Donnell MJ. Mechanisms and regulation of chloride transport in the Malpighian tubules of the larval cabbage looper Trichoplusia ni. Insect Biochem Mol Biol. 2020;116:103263. doi:10.1016/j.ibmb.2019.103263

10. Kolosov D, Piermarini PM, O'Donnell MJ. Malpighian tubules of Trichoplusia ni: Recycling ions via gap junctions and switching between secretion and reabsorption of Na+ and K+ in the distal ileac plexus. J Exp Biol. 2018;221(5). doi:10.1242/jeb.172296
11. Kolosov D, O'Donnell MJ. Blending physiology and RNAseq to provide new insights into regulation of epithelial transport: Switching between ion secretion and reabsorption. J Exp Biol. 2022;225(Suppl 1). doi:10.1242/jeb.243293
12. Agard MA, Zandawala M, Paluzzi JPV. Another fly diuretic hormone: Tachykinins increase fluid and ion transport by adult Drosophila melanogaster Malpighian 'renal' tubules. J Exp Biol. 2024;227(19). doi:10.1242/jeb.247668
13. Al-Dailami AN, Lange AB, Orchard I. The glycoprotein hormone receptor (LGR1) influences Malpighian tubule secretion rate in Rhodnius prolixus. J Exp Biol. 2024;227(24). doi:10.1242/jeb.249357
14. Patrick ML, Donini A, Zogby A, Morales C, O'Donnell MJ, Gill SS. Proton-driven sodium secretion in a saline water animal. Sci Rep. 2024;14(1):12738. doi:10.1038/s41598-024-62974-4
15. Andersen MK, Overgaard J. Maintenance of hindgut reabsorption during cold exposure is a key adaptation for Drosophila cold tolerance. J Exp Biol. 2020;223(4). doi:10.1242/jeb.213934
16. Yerushalmi GY, Misyura L, MacMillan HA, Donini A. Functional plasticity of the gut and the Malpighian tubules underlies cold acclimation and mitigates cold-induced hyperkalemia in Drosophila melanogaster. J Exp Biol. 2018;221(6). doi:10.1242/jeb.174904
17. Gáliková M, Dircksen H, Nässel DR. The thirsty fly: Ion transport peptide (ITP) is a novel endocrine regulator of water homeostasis in Drosophila. PLoS Genet. 2018;14(8). doi:10.1371/journal.pgen.1007618
18. Kandel Y, Pinch M, Lamsal M, Martinez N, Hansen IA. Exploratory phosphoproteomics profiling of Aedes aegypti Malpighian tubules during blood meal processing reveals dramatic transition in function. PLoS One. 2022;17(7). doi:10.1371/journal.pone.0271248
19. Zhao YQ, Tang YY, Hu JP, Huang YZ, Wan K, Zhang MH, et al. An aquaporin and an aquaglyceroporin have roles in low temperature adaptation of mosquitoes (Anopheles sinensis). Insect Sci. 2024;31(6):1743-55. doi:10.1111/1744-7917.13359
20. Orr SE, Collins LB, Jima DD, Buchwalter DB. Salinity-induced ionoregulatory changes in the gill proteome of the mayfly, Neocloeon triangulifer. Environ Pollut. 2023;316(Pt 2):120609. doi:10.1016/j.envpol.2022.120609
21. Andersen MK, MacMillan HA, Donini A, Overgaard J. Cold tolerance of Drosophila species is tightly linked to the epithelial K+ transport capacity of the Malpighian tubules and rectal pads. J Exp Biol. 2017;220(22):4261-9. doi:10.1242/jeb.168518
22. Andersen MK, Folkersen R, MacMillan HA, Overgaard J. Cold acclimation improves chill tolerance in the migratory locust through preservation of ion balance and membrane potential. J Exp Biol. 2017;220(3):487-96. doi:10.1242/jeb.150813
23. MacMillan HA, Nazal B, Wali S, Yerushalmi GY, Misyura L, Donini A, et al. Anti-diuretic activity of a CAPA neuropeptide can compromise Drosophila chill tolerance. J Exp Biol. 2018;221(19). doi:10.1242/jeb.185884
24. Andersen MK, Roe AD, Liu Y, Musso AE, Fudlosid S, Haider F, et al. The freeze-avoiding mountain pine beetle (Dendroctonus ponderosae) survives prolonged exposure to stressful cold by mitigating ionoregulatory collapse. J Exp Biol. 2024;227(9). doi:10.1242/jeb.247498
25. Jørgensen LB, Hansen AM, Willot Q, Overgaard J. Balanced mitochondrial function at low temperature is linked to cold adaptation in Drosophila species. J Exp Biol. 2023;226(8). doi:10.1242/jeb.245439
26. Torson AS, Yocum GD, Bowsher JH. Molecular mechanisms and trade-offs underlying fluctuating thermal regimes during low-temperature storage. Curr Opin Insect Sci. 2024;62:101160. doi:10.1016/j.cois.2024.101160
27. Helou B, Ritchie MW, MacMillan HA, Andersen MK. Dietary potassium and cold acclimation additively increase cold tolerance in Drosophila melanogaster. J Insect Physiol. 2024;159:104701. doi:10.1016/j.jinsphys.2024.104701
28. Cochran JK, Banks C, Buchwalter DB. Respirometry reveals major lineage-based differences in the energetics of osmoregulation in aquatic invertebrates. J Exp Biol. 2023;226(20). doi:10.1242/jeb.246376
29. Gáliková M, Klepsatel P. Ion transport peptide regulates energy intake, expenditure, and metabolic homeostasis in Drosophila. Genetics. 2022;222(4). doi:10.1093/genetics/iyac150

30. Huisamen EJ, Colinet H, Karsten M, Terblanche JS. Dietary salt supplementation adversely affects thermal acclimation responses of flight ability in Drosophila melanogaster. J Insect Physiol. 2022;140:104403. doi:10.1016/j.jinsphys.2022.104403