Ecological Informatics 30 (2015) 82–96

Contents lists available at ScienceDirect

Ecological Informatics

journal homepage: www.elsevier.com/locate/ecolinf

Development of a metabolite-driven daphnia ecophysiological model

Gurbir Perhar *, George B. Arhonditsis

Department of Physical and Environmental Sciences, University of Toronto, 1265 Military Trail, Scarborough, ON M1C-1A4, Canada

ARTICLE INFO

Article history:
Received 6 April 2015
Received in revised form 25 August 2015
Accepted 3 September 2015
Available online 26 September 2015

Keywords:
Daphnia
Zooplankton
Physiology
Metabolomics
Early warning system
Regime shifts

ABSTRACT

One of the founding principles of modern aquatic ecology is that human-induced perturbations in the autotroph-herbivore interface have the potential to affect ecological processes at higher trophic levels. Thus, zooplankton's physiological state can be an early warning sign of broader impairments of aquatic ecosystems. Based on this reasoning, the micro-crustacean *Daphnia* is often identified as a keystone freshwater species, but its bioenergetic motivations and physiological priorities remain only partially understood. Using a bioenergetically explicit ecophysiological approach, we model how trade-offs in resource allocation can shape a daphnid's growth. Our multi-faceted hierarchical approach to metabolite utilization challenges the popular paradigm of elemental stoichiometry being the primary regulatory factor of algal food quality. We examine the post-gut bioenergetic ramifications of an unbalanced diet, showing that animal growth can be significantly compromised by the elevated energetic requirements of homeostasis. Our modeling framework offers an excellent stepping-stone to connect zooplankton physiological processes with the signals of external stressors, and subsequently evaluate the patterns of mass and energy flow at an ecosystem scale. The proposed microscopic-to-macroscopic strategy will likely offer a new prospect towards the development of early warning systems for the management of freshwater resources.

1. Introduction

Freshwater ecosystems are subject to constant changes in their physical properties and water chemistry, invasion of exotic species, and over-harvesting of fisheries (Altshuler et al., 2011). To maintain the health and integrity of freshwater environments, the establishment of early warning systems has been proposed to reliably detect imminent ecosystem organizational discontinuities (or other non-linear state shifts) to react strategically ahead of time (Scheffer et al., 2001). The concept of an early warning system is comprised of three distinct phases: i) identification and collection of weak signals; ii) analysis of trends in space and time; and iii) formulation of appropriate management responses (Scheffer and Carpenter, 2003). In this context, weak signals are unstructured, fragmented, incomplete data points that require deep analysis to be articulated into valuable information (see Mendonca et al., 2015). In freshwater environments, the sheer number of signals to process makes the development of early warning systems challenging, but shifting the focus to keystone species can offer a pragmatic strategy to move forward. The keystone species concept was first introduced by Paine (1969), stating that the presence of certain species is crucial in maintaining the organization and diversity of ecological communities. Namely, this concept postulates that there are species exerting direct and indirect influences on biotic assemblages disproportionately and thus can profoundly impact the overall food web structure and functioning (Garibaldi and Turner, 2004; Libralato et al., 2006; Paine, 1995). Nonetheless, the current monitoring methods are disconnected from the stress levels of individual organisms, and usually focus on larger-scale ecological patterns that may not be sensitive indicators of potential ecosystem regime shifts (Scheffer et al., 2012).

Herbivorous zooplankton transform plant material into animal tissue, resulting from their role as intermediaries between primary production and secondary consumption in aquatic food webs. Pelagic fish, crustaceans, mollusks, and mammals depend on zooplankton both directly and indirectly (Gajbhiye, 2002). Their large community density, relatively short life spans, high phenotypic diversity, and ability to exert grazing pressure on algae lend them to be used as indicator organisms for physical, chemical, and biological processes in aquatic ecosystems. Several studies identify zooplankton of the genus *Daphnia* as keystone herbivores (Altshuler et al., 2011; Colbourne et al., 2011; Miner et al., 2012; Persson et al., 2007; Seda and Petrusek, 2011; Sperfeld and Wacker, 2009; Straile et al., 2012; Van Doorslaer et al., 2009; Wagner and Benndorf, 2007). *Daphnia* spp. are effective filter feeders with high grazing impacts on phytoplankton biomass and species composition (Martin-Creuzburg et al., 2005). They are a preferred food choice for both vertebrate and invertebrate predators, stemming largely from their nutritious composition, relatively large size, and limited ability to evade predation (Lampert, 1987; Miner et al., 2012). Thus, daphnids are an integral link between primary production and higher trophic levels. *Daphnia* spp. populations are also very sensitive to modern toxicants in the environment, and are thus used to assess the ecological impacts of environmental change (Colbourne et al., 2011). Natural stressors include bacterial infections, predation and parasitism,

* Corresponding author.
E-mail address: g.perhar@mail.utoronto.ca (G. Perhar).

http://dx.doi.org/10.1016/j.ecoinf.2015.09.002
1574-9541/

synthetic hormones, diet variation, ultraviolet radiation, hypoxia, acidity, salinity, and low ambient calcium levels (Altshuler et al., 2011). These sensitivities are offset with *Daphnia* spp.'s high phenotypic plasticity. Specifically, they have the ability to alter diurnal vertical migration patterns and develop extensive morphological defense features to avoid predators, and can adjust hemoglobin levels in response to falling oxygen availability (Colbourne et al., 2011).

Zooplankton experimental methodologies often involve the use of micro- and mesocosms, combined with controlled changes in ambient conditions (e.g., Kurbatova, 2005; Loureiro et al., 2013; Patterson et al., 2002; Rothhaupt, 1997; Sorf et al., 2015; Wang et al., 2009). The metrics produced can be reasonable proxies of ecosystem scale dynamics, but offer limited insights into their mechanistic underpinning. For example, studies analyzing the toxicity of various metals (Biesinger and Christensen, 1972), parasites (Aalto et al., 2013), and algae (Hietala et al., 1995) on *Daphnia* spp. report survival rates, reproduction rates, and life history progression as toxicity metrics. A drawback of these studies, however, is the "black-boxed" nature of the inference drawn, in the sense that patterns are established but the physiological sequence of events driving these patterns is not. More recently, gene mapping has opened up a new avenue of investigation. Because *Daphnia* spp. ecology is fairly well understood, access to its genomic sequences allows for detailed investigation of environmental influences on gene functions (Colbourne et al., 2011). Jansen et al. (2013), for example, found distinct, time-dependent transcriptional expressions in *Daphnia magna* subject to differential stresses. In the post genomic era, methodological advances are driven by technologies allowing functions of both cells and whole organisms to be explored at the molecular level (Whitfield et al., 2004).

Changes in tissues and biological fluids are indicative of an animal's well being (Micholson and Lindon, 2008), and provide a comprehensive molecular view of cellular control mechanisms (Whitfield et al., 2004). Metabolomics is the study of naturally occurring, low molecular weight organic metabolites within cells, tissues, and biofluids (Griffiths, 2007). Metabolomic measurements can be mechanistically related to higher levels of biological organization (Bundy et al., 2009). Metabolomics provide an integrated view of biochemistry in complex organisms, as opposed to the traditional approach associated with systems biology, whereby interactions between genes, proteins, and metabolites in individual cell types are investigated (Nicholson et al., 1999). The problem with the latter approach are the different time scales at which each level of biological organization (i.e., genomics, gene expression, protein expression, and metabolism) operate, making it difficult to find causal linkages (Nicholson et al., 1999). The application of metabolomics to characterize organism interactions with their environments is called environmental metabolomics, and these interactions can be studied from individuals to populations (Bundy et al., 2009).

If the objective of a modeling exercise is to explain large scale patterns rather than describe them, the patterns need to be built up from processes (Royle and Dorazio, 2008). In zooplankton modeling, this inevitably requires consideration of physiological processes. An early example of this strategy came from Sjoberg (1980), who assumed zooplankton to vary its feeding strategy in response to gut contents. Food particles in the gut were treated as a queue, in that digestion was a service given only to the food item holding first position, and consequently the digestive process was the growth limiting factor instead of the food capture and ingestion. Dynamic Energy Budget (DEB) models are a relatively recent concept, aiming to distinguish the metabolic organization of individual organisms (Kooijman, 2001). DEB theory provides a framework to build process-based models for organism life cycles (Jager et al., 2013), and explain body-size scaling relationships of natural history parameters that can otherwise be difficult to comprehend (Kooijman, 2001). DEB models track structural and storage somatic constructs across life stages. In a similar context, Perhar et al. (2012a, 2012b) attempted to integrate zooplankton physiology into a fully functional ecosystem model. Namely, zooplankton dynamics were driven from physiological processes including molt turnover, biomass turnover, hormone production, regulatory release, and internal nutrient and highly unsaturated fatty acid reserves. These processes were shown to heavily influence the producer-consumer interface, while fundamentally altering the nature of ecosystem feedback loops (Perhar et al., 2012c).

Perhar et al. (2012c) questioned the validity of extreme modeling results in the literature that are rarely (if ever) observed in nature. In particular, the explicit consideration of intra-organism processes into a conventional plankton model phased out the emergence of oscillatory behavior associated with the paradox of enrichment (Rosenzweig, 1971). Similarly, Fussmann and Heber (2002) argued that while very simple mathematical frameworks can display chaotic dynamics, natural food webs likely possess architectural properties that can intrinsically minimize the likelihood to observe these patterns in the real world. Thus, linking processes at the organismal level with large-scale food web patterns can maximize the utility of ecosystem models in a management context.

In this study, our key objective is to utilize *Daphnia* spp. metabolomic data to formulate a mechanistic individual-based model. By linking each metabolite (or congener) to a physiological process, internal concentrations can shed light on the individual's health. Because it can be easily linked to management-oriented ecosystem models, the overarching benefit of our individual-based physiological model is its potential to serve as a device for detecting early warning signs. Our proposed "microscopic-to-macroscopic" strategy will provide a mechanistic look into the bioenergetic motivations of daphnids, and potentially offer a new methodological tool for water resource management.

2. Methodology

Our zooplankton physiology framework is intended to plug into mass balance ecosystem models to enhance the realism of zooplankton dynamics. We have built on the foundation of Anderson et al. (2005), who modeled a stoichiometrically explicit nutrient regulation strategy in *Daphnia*. The authors considered the carbon (*C*), nitrogen (*N*), and phosphorus (*P*) contents of ingested food, with each congener playing a unique somatic role. By considering a post-gut regulation mechanism, Anderson et al. (2005) were able to illustrate the delicate balance between food quantity and food quality. Subsequent advances in the modeling literature have also illustrated this balance (Perhar et al., 2012b, 2012c). Perhar et al. (2012a) built on this approach and considered the highly unsaturated fatty acids (*HUFAs*) eicosapentaenoic acid (*EPA*) and docosahexaenoic acid (*DHA*), in addition to *N* and *P* to quantify somatic growth in their zooplankton submodel. We continue here along a similar trajectory, with an expanded domain considering 14 congeners (see Table 1). Each congener is a proxy for a physiological process. Using congener saturation measures, we quantify the potential investment for each physiological process considered. Congeners are gathered through food intake, which is assumed to be a fixed rate (*GRAZ*). We employ a food quality index (*FQ*) in a fashion similar to Perhar et al. (2012a), whereby morphological and toxicological features are defined by a single index. The food quality index, along with parameters accounting for thermodynamic losses (α_{C1} and α_{C2}; see Table 1) quantify carbon assimilation efficiency (*AE*). The product of assimilation efficiency and grazing rate yields assimilated carbon (*AC*).

$$AE = \frac{\alpha_{C1} \cdot FQ}{\alpha_{C2} + FQ} \tag{1}$$

$$AC = GRAZ \cdot AE. \tag{2}$$

Assimilated congener ($A_{S_i:C}$) is calculated by multiplying *AC* with food congener to carbon ratios (see Table 3). Assimilated congeners are added to existing somatic pools, from where they are mobilized for physiological use.

$$A_{S_i:C} = AC \cdot food_{S_i:C} \tag{3}$$

Table 1
Model parameters pertaining to zooplankton growth, somatic congener concentrations, and physiological energetic fractionations. Parameter values denoted by * indicate experimental variation.

Symbol	Value	Unit	Description
TRY_{som}	8.69	μg TRY mg C^{-1}	Somatic tryptophan to carbon ratio
TYR_{som}	9.47	μg TRY mg C^{-1}	Somatic tyrosine to carbon ratio
$CARB_{som}$	52.11	μg CARB mg C^{-1}	Somatic carbohydrate to carbon ratio
FAT_{som}	52.23	μg FAT mg C^{-1}	Somatic saturated fatty acid to carbon ratio
$PROT_{som}$	63.27	μg PROT mg C^{-1}	Somatic protein to carbon ratio
CLS_{som}	10.12	μg CLS mg C^{-1}	Somatic cholesterol to carbon ratio
CHO_{som}	3.87	μg CHO mg C^{-1}	Somatic choline to carbon ratio
EPA_{som}	9.85	μg EPA mg C^{-1}	Somatic eicosapentaenoic acid to carbon ratio
DHA_{som}	1.12	μg DHA mg C^{-1}	Somatic docosahexaenoic acid to carbon ratio
CYS_{som}	6.93	μg CYS mg C^{-1}	Somatic cysteine to carbon ratio
GLY_{som}	2.07	μg GLY mg C^{-1}	Somatic glycine to carbon ratio
GA_{som}	17.23	μg GA mg C^{-1}	Somatic glutamic acid to carbon ratio
P_{som}	3.50	μg P mg C^{-1}	Somatic phosphorus to carbon ratio
N_{som}	31.16	μg N mg C^{-1}	Somatic nitrogen to carbon ratio
$GRAZ$	0.85	day^{-1}	Grazing rate
α_{C1}	0.9	Unitless	Thermodynamic constraint 1
α_{C2}	0.03	Unitless	Thermodynamic constraint 2
G_{MAX}	0.65	day^{-1}	Maximum growth rate
E_h	0.75	Unitless	Use efficiency of energy allocated to anabolism and reproduction
b	5	day^{-1}	Rate of congener turnover at supersaturation
c	2	Unitless	Increase of turnover rate with increasing supersaturation
N_{NEURO}	0.5	Unitless	Fraction of nitrogen for neurotransmitter synthesis
N_{ANA}	0.5	Unitless	Fraction of nitrogen for growth
P_{ENERGY}	0.33	Unitless	Fraction of phosphorus for energetics
P_{MAINT}	0.33	Unitless	Fraction of phosphorus for maintenance
P_{ANA}	0.33	Unitless	Fraction of phosphorus for growth
FAT_{ENERGY}	0.5	Unitless	Fraction of fat for energetics
FAT_{MAINT}	0.5	Unitless	Fraction of fat for maintenance
CLS_{MAINT}	0.5	Unitless	Fraction of cholesterol for maintenance
CLS_{ANA}	0.5	Unitless	Fraction of cholesterol for growth
EC_{brkd_1}	*	Unitless	Fraction of total energy for maintenance
EC_{brkd_2}	*	Unitless	Fraction of total energy for anabolism
EC_{brkd_3}	*	Unitless	Fraction of total energy for reproduction
OSM_{brkd_1}	0.25	Unitless	Fraction of maintenance energy allotted to Phosphorus
OSM_{brkd_2}	0.25	Unitless	Fraction of maintenance energy allotted to choline
OSM_{brkd_3}	0.25	Unitless	Fraction of maintenance energy allotted to cholesterol
OSM_{brkd_4}	0.25	Unitless	Fraction of maintenance energy allotted to fat
WAS_{brkd_1}	0.33	Unitless	Fraction of waste management energy allotted to glycine
WAS_{brkd_2}	0.33	Unitless	Fraction of waste management energy allotted to glutamic acid
WAS_{brkd_3}	0.33	Unitless	Fraction of waste management energy allotted to cysteine
ANA_{brkd_1}	0.33	Unitless	Fraction of anabolic energy allotted to Phosphorus
ANA_{brkd_2}	0.33	Unitless	Fraction of anabolic energy allotted to Nitrogen
ANA_{brkd_3}	0.33	Unitless	Fraction of anabolic energy allotted to cholesterol
REP_{brkd_1}	0.5	Unitless	Fraction of reproductive energy allotted to EPA
REP_{brkd_2}	0.5	Unitless	Fraction of reproductive energy allotted to DHA
$NeuroRate$	0.1	day^{-1}	Neurological congener mobilization rate
$MobRate$	0.1	day^{-1}	Energetic congener mobilization rate
$TurnoverRate$	0.1	day^{-1}	Supersaturated congener turnover rate

2.1. Physiological processes

The list of physiological processes modeled was devised around available (or soon to be available) lab data. Processes include: i) neurological function, ii) energetics, iii) osmoregulatory maintenance, iv) waste management, and v) growth. We believe this strikes a pragmatic balance for a first approximation, and more processes (or more highly resolved processes) can be accounted for in subsequent studies. We use tyrosine (*TYR*), tryptophan (*TRY*), and a fraction of *N* (N_{NEURO}; see Table 1 for all physiological fractions and rates) as proxies for neurological function. *TYR* is a non-essential amino acid (it can be synthesized from phenylalanine), while *TRY* is an essential amino acid. Both are present in proteins used in signal transduction. More specifically, they are important precursors for neurotransmitters and hormones – namely octopamine and serotonin, respectively – both of which can be further transformed. We consider an array of high caloric, energy carrying compounds for energetic functions, including carbohydrates (combining glucose and maltose measurements; *CARB*), dietary proteins (*PROT*), saturated fatty acids (*FAT*), and a fraction of ingested *P* (P_{ENERGY}). We have chosen to separate somatic growth (anabolism from here on out) from reproductive growth (see following section for physiological priorities), but acknowledge that distinguishing somatic growth (coinciding with molting) from reproductive growth (i.e., releasing eggs) may not be a straightforward process. We have chosen to use fractions of cholesterol (CLS_{ANA}), *N* (N_{ANA}) and *P* (P_{ANA}), to reflect the anabolic investments of the daphnid. *CLS* is an organic molecule used in the production of growth hormones and steroids (Heffner and Schust, 2010), while both *N* and *P* are required for nucleic acid synthesis (Alberts et al., 2002). We characterize reproductive investments using *EPA* and *DHA*, both of which are amassed in daphnid eggs. Both *EPA* and *DHA* are also required for sex hormone production, and a high abundance of these molecules can indicate high reproductive potential. We link osmoregulatory maintenance costs primarily to phospholipid turnover. Choline (*CHO*) is a precursor to phosphatidylcholine (a phospholipid), and serves as a surrogate for osmoregulatory maintenance. Fractions of *CLS* (CLS_{MAINT}), *P* (P_{MAINT}), and *FAT* (FAT_{MAINT}) are also required for lipid raft, hydrophilic head, and hydrophobic tail formation, respectively. Glutathione is a molecule strongly associated with detoxification in aquatic crustaceans (Billiard

Table 2
Criteria to consider when selecting/creating an ecological indicator; paraphrased from Doren et al. (2009).

	Criteria	Our Framework
a.	Is indicator relevant to the ecosystem?	Yes. The literature contains numerous studies using *Daphnia* spp. as indicator/sentinel species in freshwater ecosystems.
b.	Do indicator response dynamics represent the entire system, or a portion of the system?	One of the founding concepts of our framework is that the quantification of the fitness of *Daphnia* spp. at the molecular level can shape food web dynamics. The integration with an ecosystem model offers a platform to examine the broader ramifications of shifts into the herbivorous community and thus formulate risk assessment statements.
c.	Is indicator implementation feasible? Is the indicator measurable?	The advent of metabolomics offers an unbiased view of the complex interactions that characterize functioning biochemical networks. The incorporation of these measurements in regular monitoring programs will make the implementation of the proposed framework straightforward.
d.	Is indicator sensitive to system drivers? Is it predictable?	Yes. *Daphnia* spp. being keystone species are, by definition, highly sensitive to system drivers.
e.	Are indicator trends interpretable in common language?	Yes. The *QM* value provides a snapshot of somatic congener status and represents a first attempt to create an easily interpretable indicator.
f.	Can indicator trends be misleading?	The probability of Type I or Type II errors cannot be ruled out. The indicator may not capture higher level confounding factors that disconnect the health of *Daphnia* spp. from the actual ecosystem state. Coupling our eco-physiological model with the food web dynamics offers a tool to examine the likelihood of alternative ecological scenarios, but the consideration of other keystone species at higher trophic levels may be necessary.
g.	Can indicator trend be unrelated to restoration activity?	On its own, the *QM* may not capture dynamics unrelated to restoration activities, but coupling with a food web model may yield additional insights. In any event, any systematic trends in *Daphnia* spp.'s metabolomic signature warrant further investigation of the underlying causal factors.
h.	Is indicator scientifically defensible?	Yes. Our eco-physiology model is mechanistically built from theory, and the metabolomic data driving our framework offers a granularity not previously feasible.
i.	Can clear, measurable targets be set?	Yes. Targets can be set for each congener, based on where they reside on the *QM*.
j.	Does indicator have specificity (i.e., a strong interpretable effect of a stressor)?	Yes. The literature contains many studies focusing on *Daphnia's* response to various toxins (Hietala et al., 1995), food quantity (Guisande and Gliwicz, 1992), and food quality (Wacker and Martin-Creuzburg, 2007). The culmination of these investigations tend to be a single phenotypic trait (e.g., mass, growth rate, egg production rate). Our framework attempts to base the decision making process on a multi-dimensional metric, effectively connecting molecular level responses and phenotype variability to multiple external stressors.

et al., 2008). It is synthesized from cysteine (*CYS*), glutamic acid (*GA*), and glycine (*GLY*). These three amino acids serve as surrogates for detoxification and waste management requirements.

2.2. Physiological priorities

Physiological priorities also need to be defined. That is, the rules against which the physiological processes are executed within the individual require formal structure. Our framework runs on logic similar to that used by Perhar et al. (2012a), whereby each congener pool's saturation level (INT) is quantified against minimum and optimum values, and these saturation measures drive subsequent model dynamics (see Eq. (4)). When introducing the concept of intracellular resource quotas in phytoplankton, Droop (1968) used minimum and maximum resource bounds. The minimum bound represented the absolute minimum cellular requirement for the cell to survive, and the maximum reflected the volumetric storage constraints. In extending the intracellular quota model to zooplankton, Perhar et al. (2012a) retained the minimum resource bound (now reflecting the absolute minimum resource required for the organism to maintain its bodily functions at zero growth), but argued that a resource maxima was unrealistic. Instead, they opted for an optimal resource level, at which (all else being equal) maximum growth is achieved. The difference in notation (maximum resource vs. optimal resource) is not trivial, and it carries key physiological and ecological weight. From a structural point of view, a zooplankter is significantly more complex than an algal cell, and as such, the volumetric limitations to storage in algae (dictated by vacuole volume) do not necessarily apply to zooplankton. In addition, zooplankton (like many other animals) vary in size, related to age and diet. Thus, where an algal cell realizes its volumetric resource maxima, a zooplankter may synthesize additional storage reserves. An optimal resource concentration suggests limitations to growth both below and above this threshold, an idea discussed but not explored by Perhar et al. (2012c). The current framework will base model parameter values on metabolome observations from a wet lab. As such, we have created somatic minimum and optimum bounds by taking 10% and 125% of the provided average somatic congener values (see Table 1 for default somatic congener values used).

Table 3
Congener availability and morphological food quality of high and low quality algae. Experiments not specifying algal parameterization defaulted to high quality. Congener units are μg mg C^{-1}, *FQ* is unitless. The primary differences between high and low quality algae are neurocongener concentrations, energetic congener concentrations, HUFA concentrations, and morphological features.

Parameter	High	Low
TRY	19.60	14.70
TYR	23.38	17.53
CARB	99.40	112.0
FAT	70.82	101.17
PROT	89.92	25.15
CHO	5.80	5.80
CHL	11.96	11.96
EPA	12.07	1.81
DHA	5.80	0.078
CYS	10.15	10.15
GLY	2.36	2.36
GA	17.73	14.73
P	3.25	3.25
N	51.45	51.45
FQ	0.9	0.025

$$S_{i_{SAT}} = \frac{S_{i_{INT}} - S_{i_{MIN}}}{S_{i_{OPT}} - S_{i_{MIN}}} \quad (4)$$

2.2.1. Neurological function

We assume an internal physiological hierarchy, such that neurological congener saturation drives organism energetics, and energy is distributed across the remaining physiological processes, which have their own hierarchy (see Fig. 1). Neurological saturation level ($NEUR_{SAT}$) is a measure reflecting an individual's capacity to synthesize neurotransmitters, and subsequently control and regulate all "downstream" physiological functions. *TRY* and *TYR* are the amino acid precursors of dopamine and octopamine, and neurological capacity is approximated using the least saturated pool; neurological *N* requirements are assumed to be in excess. By mediating energetic expenditure with neurological capacity, we ensure that daphnid response is not

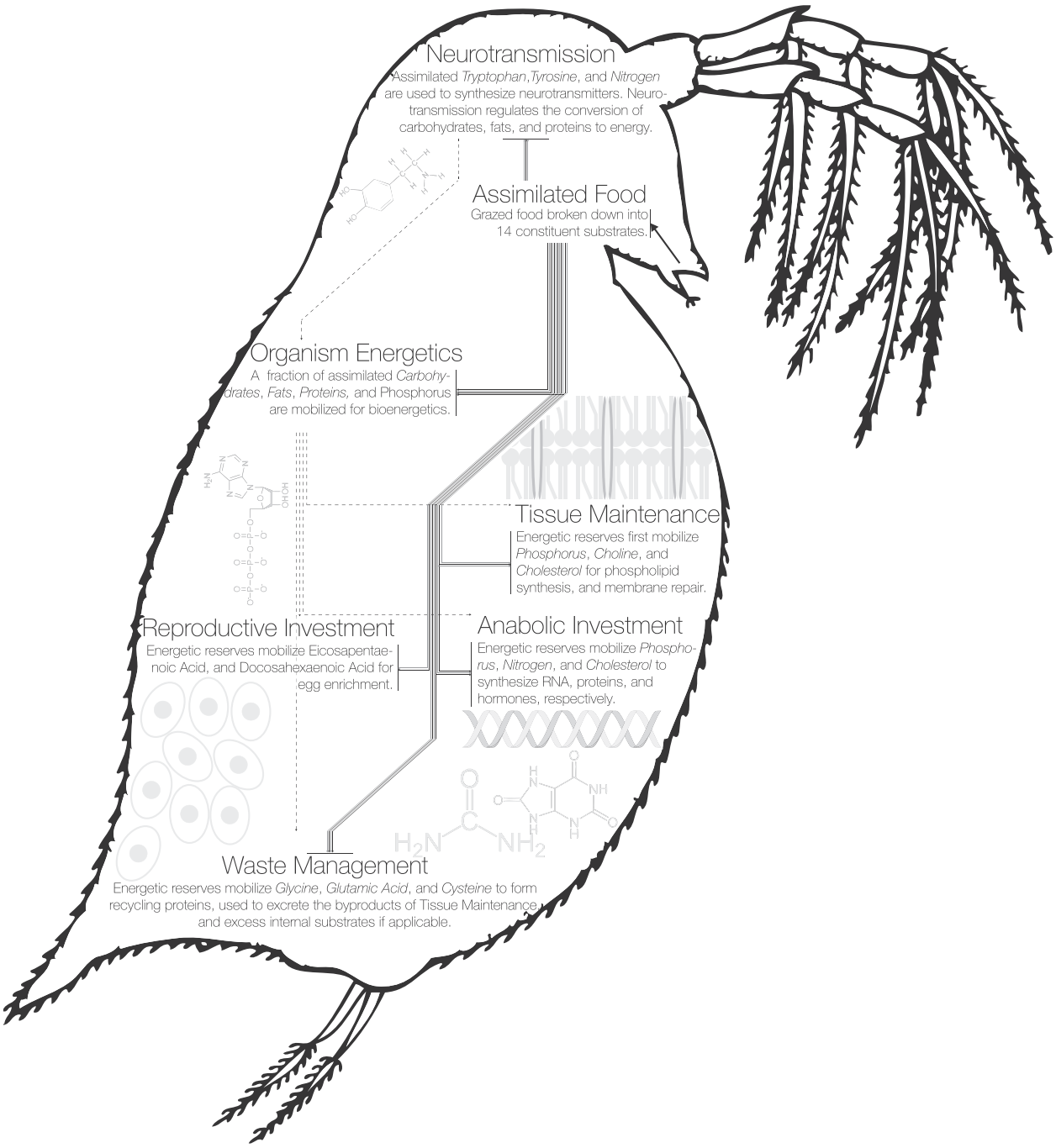


Fig. 1. Modeled physiological processes and priorities. Solid lines terminating in arrowheads represent congener fates, while dashed lines represent the flow of energy.

driven solely by either food quality or quantity. In other words, the individual cannot make use of rich food quality (for maintenance, anabolism, and reproduction) unless it is balanced with quantity (i.e., energetics), but energetic capacity, in turn, cannot be optimized with suboptimal neurological conditions.

$$NEUR_{SAT} = min(TRY_{SAT}, TYR_{SAT}) \tag{5}$$

Neurological congener mobilization is rate controlled (*NeuroRate*), thus, the quantities of *TRY*, *TYR*, and *N* mobilized for neurotransmitter synthesis are:

$$TRY_{M_{neuro}} = TRY_{INT} \cdot TRY_{SAT} \cdot NeuroRate \tag{6}$$

$$TYR_{M_{neuro}} = TYR_{INT} \cdot TYR_{SAT} \cdot NeuroRate \tag{7}$$

$$N_{M_{neuro}} = N_{INT} \cdot N_{NEURO} \cdot NeuroRate. \tag{8}$$

2.2.2. Energetics

The fraction of metabolic compounds mobilized for energy production ($CARB_{MOB_M}$, FAT_{MOB_M}, and $PROT_{MOB_M}$) is governed by neurological capacity, and a mobilization rate (*MobRate*). The mobilized metabolic compounds are converted to energy units ($CARB_{MOB_E}$, FAT_{MOB_E}, and $PROT_{MOB_E}$) using yield per unit mass conversion factors ($CARB_{YIELD}$, FAT_{YIELD}, and $PROT_{YIELD}$; see Table 4). These energies are summed to provide total energetic capacity (*EC*). Note that only a fraction of assimilated *FAT*

Table 4
Energetic yields of carbohydrates, fat, and proteins, taken from the Food and Agriculture Organization of the United Nations' website (2014).

Parameter	Value	Unit
$CARB_{YIELD}$	4	$\frac{kcal}{g}$
FAT_{YIELD}	9	$\frac{kcal}{g}$
$PROT_{YIELD}$	4	$\frac{kcal}{g}$

(FAT_{ENERGY}) is used for energy; the remainder is used for maintenance. Total energetic capacity is further modulated by the fraction of P allocated to energetics (P_{ENERGY}), reflecting the ATP cycle.

$$CARB_{MOB_M} = NEUR_{SAT} \cdot CARB_{INT} \cdot MobRate \quad (9)$$

$$CARB_{MOB_E} = CARB_{MOB_M} \cdot CARB_{YIELD} \quad (10)$$

$$FAT_{MOB_M} = NEUR_{SAT} \cdot FAT_{INT} \cdot FAT_{ENERGY} \cdot MobRate \quad (11)$$

$$FAT_{MOB_E} = FAT_{MOB_M} \cdot FAT_{YIELD} \quad (12)$$

$$PROT_{MOB_M} = NEUR_{SAT} \cdot PROT_{INT} \cdot MobRate \quad (13)$$

$$PROT_{MOB_E} = PROT_{MOB_M} \cdot PROT_{YIELD} \quad (14)$$

$$P_{MOB_M} = P_{INT} \cdot P_{ENERGY} \cdot MobRate \quad (15)$$

$$EC = (CARB_{MOB_E} + FAT_{MOB_E} + PROT_{MOB_E}) \cdot P_{SAT} \cdot P_{ENERGY}. \quad (16)$$

The developmental priorities of an individual are defined by the energetic breakdown vector (EC_{brkd}). The three components describe the energetic allocations for osmoregulatory maintenance (OSM), somatic growth and anabolic investment (ANA), and reproductive investment (REP). The vector does not sum to 1, as ANA and REP are nested under OSM. That is, all remaining energy after maintenance and recycling (see Waste Management section) is divided between anabolic and reproductive investment. Thus, EC_{brkd} defines an individual's physiological priorities/strategy:

$$EC_{brkd} = [OSM, ANA, REP]. \quad (17)$$

2.2.3. Osmoregulatory maintenance

The energy allocated to osmoregulatory maintenance (E_{OSM}) is used to mobilize P, CHO, CLS, and FAT for the synthesis and repair of phospholipids and membranes. An additional parameter vector (OSM_{brkd}) is required to define the allotment of energy across each substrate required for this process:

$$E_{OSM} = EC \cdot EC_{brkd_1} \quad (18)$$

$$OSM_{brkd} = [P, CHO, CLS, FAT]. \quad (19)$$

The quantities of mobilized P, CHO, CLS, and FAT for osmoregulatory requirements are determined using congener specific energetic allocations, and congener activation energies ($I_{A.E.}$; see Table 5), and are further modulated using the internal congener concentration. Each of P, CLS, and FAT are subject to a maintenance fractionation (e.g., P_{MAINT}), whereby a preallocated fraction of the respective internal reserves is used for maintenance. In our current framework, CHO is a monofate congener required only for maintenance processes, and thus, does not require a fractionation parameter.

$$P_{M_{osm}} = \frac{E_{OSM} \cdot OSM_{brkd_1}}{P_{A.E.}} \cdot P_{SAT} \cdot P_{MAINT} \quad (20)$$

Table 5
Congener activation energies and sources.

Congener	Value	Unit	Source
CHL	55 − 65	$\frac{kJ}{mol}$	Filippov et al. (2003)
CHO	16	$\frac{kcal}{mol}$	Plagemann (1971)
EPA	36.5	$\frac{kJ}{mol}$	Cantrell and Walker (2009)
DHA	30	$\frac{kJ}{mol}$	Yoshii et al. (2006)
CYS	29.4 − 32.2	$\frac{kcal}{mol}$	Nurnsten (2005)
GA	34.4	$\frac{kJ}{mol}$	Wu et al. (2000)
GLY	24 − 30	$\frac{kcal}{mol}$	Aliev and Harris (2004)
N	36.8	$\frac{kcal}{mol}$	Ang (1953)
P	40	$\frac{kJ}{mol}$	Torrent (1991)

$$CHO_{M_{osm}} = \frac{E_{OSM} \cdot OSM_{brkd_2}}{CHO_{A.E.}} \cdot CHO_{SAT} \quad (21)$$

$$CLS_{M_{osm}} = \frac{E_{OSM} \cdot OSM_{brkd_3}}{CLS_{A.E.}} \cdot CLS_{SAT} \cdot CLS_{MAINT} \quad (22)$$

$$FAT_{M_{osm}} = \frac{E_{OSM} \cdot OSM_{brkd_4}}{FAT_{A.E.}} \cdot FAT_{SAT} \cdot FAT_{MAINT} \quad (23)$$

2.2.4. Waste management

A dynamic allotment of energy is required for waste management processes. This energy is used to excrete byproducts of osmoregulatory maintenance (at a 1:1 ratio), and excess substrates (see Eq. (26)), by mobilizing GLY, GA, and CYS. An additional parameter vector (WAS_{brkd}) is required to define the allotment of energy across the substrates required for this process:

$$E_{WAS} = E_{OSM} + \sum T_{S_i:C} \quad (24)$$

$$WAS_{brkd} = [GLY, GA, CYS]. \quad (25)$$

When somatic congener concentrations exceed their respective optimums, the organism is forced to allocate energy to excrete excess substrates, which in turn lowers the energy remaining for growth. This explicitly addresses the concerns of Perhar et al. (2012b), who argued that the use of optimal congener concentrations requires penalized performance past the point of saturation. If the saturation level of a given congener is less than 1, no substrate is subject to regulatory turnover. But once the optimal concentration is exceeded (i.e., supersaturation), a rate controlled portion of the excess substrate ($T_{S_i:C}$) is recycled back into the water column following a sigmoid pattern. Our model uses the Gompertz function to depict the rigidity of homeostasis, whereby the turned over mass increase as the excess material increases:

$$T_{S_i:C} = TurnoverRate \cdot e^{-b^{-c\left(\frac{S_{i_{INT}} - S_{i_{OPT}}}{S_{i_{OPT}}}\right)}} \cdot S_{i_{A.E.}} \quad (26)$$

where b specifies the turnover rate once the optimal concentration is exceeded; c sets the increase of the turnover rate as the degree of supersaturation for a particular congener increases. Congeners mobilized for the recycling of maintenance byproducts are quantified as follows:

$$GLY_{M_{was}} = \frac{E_{WAS} \cdot WAS_{brkd_1}}{GLY_{A.E.}} \cdot GLY_{SAT} \quad (27)$$

$$GA_{M_{was}} = \frac{E_{WAS} \cdot WAS_{brkd_2}}{GA_{A.E.}} \cdot GA_{SAT} \quad (28)$$

$$CYS_{M_{was}} = \frac{E_{WAS} \cdot WAS_{brkd_3}}{CYS_{A.E.}} \cdot CYS_{SAT}. \quad (29)$$

2.2.5. Growth

The energy remaining (ER) after osmoregulatory maintenance, byproduct recycling, and congener turnover (where applicable) is distributed to growth processes (anabolic and reproductive investments), in accordance with the energetic breakdown vector (EC_{brkd}).

$$ER = EC - E_{OSM} - E_{WAS} \tag{30}$$

$$E_{ANA} = ER \cdot EC_{brkd_2} \tag{31}$$

$$E_{REP} = ER \cdot EC_{brkd_3}. \tag{32}$$

The energetic allotment for anabolic investment is used to mobilize P, N, and CLS to form growth related molecules (e.g., RNA, proteins, and hormones). An additional parameter vector (ANA_{brkd}) is required to define the allotment of energy across the substrates required for this process. Further, each substrate is fractionated, indicating the portion reserved for somatic growth.

$$ANA_{brkd} = [P, N, CLS] \tag{33}$$

Each congener required for anabolic investment is multifated, and as such, requires explicit fractionation for resource allocation (e.g., P_{ANA}). Congeners mobilized for anabolic investment are quantified as follows:

$$P_{M_{ana}} = \frac{E_{ANA} \cdot ANA_{brkd_1}}{P_{A.E.}} \cdot P_{SAT} \cdot P_{ANA} \tag{34}$$

$$N_{M_{ana}} = \frac{E_{ANA} \cdot ANA_{brkd_2}}{N_{A.E.}} \cdot N_{SAT} \cdot N_{ANA} \tag{35}$$

$$CLS_{M_{ana}} = \frac{E_{ANA} \cdot ANA_{brkd_3}}{CLS_{A.E.}} \cdot CLS_{SAT} \cdot CLS_{ANA}. \tag{36}$$

The energy allotted for reproductive investment is used to mobilize EPA and DHA for sex hormone production and egg enrichment. An additional parameter vector (REP_{brkd}) is required to define the allotment of energy across the substrates required for this process.

$$REP_{brkd} = [EPA, DHA] \tag{37}$$

Congeners mobilized for reproductive investments are quantified as follows:

$$EPA_{M_{rep}} = \frac{E_{REP} \cdot REP_{brkd_1}}{EPA_{A.E.}} \cdot EPA_{SAT} \tag{38}$$

$$DHA_{M_{rep}} = \frac{E_{REP} \cdot REP_{brkd_2}}{DHA_{A.E.}} \cdot DHA_{SAT}. \tag{39}$$

Organism growth rate (G) is a fraction of the maximum growth rate (G_{MAX}). The fraction compares the amount of energy the organism has remaining after maintenance and waste management, against the maximum potential energy that could have been used for growth:

$$G = \frac{E_{ANA} + E_{REP}}{E_{ANA} + E_{REP} + E_h} \cdot G_{MAX} \tag{40}$$

where the parameter E_h characterizes the use efficiency of the energy allotted to anabolism and reproduction.

2.3. Governing equations

Tying the individual's physiological processes and priorities together, are the model's governing equations:

$$\frac{dTRY_{INT}}{dt} = A_{TRY:C} - TRY_{M_{neuro}} - T_{TRY:C} - TRY_{INT} \cdot G \tag{41}$$

$$\frac{dTYR_{INT}}{dt} = A_{TYR:C} - TYR_{M_{neuro}} - T_{TYR:C} - TYR_{INT} \cdot G \tag{42}$$

$$\frac{dCARB_{INT}}{dt} = A_{CARB:C} - CARB_{MOB_M} - T_{CARB:C} - CARB_{INT} \cdot G \tag{43}$$

$$\frac{dFAT_{INT}}{dt} = A_{FAT:C} - (FAT_{MOB_M} + FAT_{M_{osm}}) - T_{FAT:C} - FAT_{INT} \cdot G \tag{44}$$

$$\frac{dPROT_{INT}}{dt} = A_{PROT:C} - PROT_{MOB_M} - T_{PROT:C} - PROT_{INT} \cdot G \tag{45}$$

$$\frac{dN_{INT}}{dt} = A_{N:C} - (N_{M_{neuro}} + N_{M_{ana}}) - T_{N:C} - N_{INT} \cdot G \tag{46}$$

$$\frac{dP_{INT}}{dt} = A_{P:C} - (P_{MOB_M} + P_{M_{osm}} + P_{M_{ana}}) - T_{P:C} - P_{INT} \cdot G \tag{47}$$

$$\frac{dCLS_{INT}}{dt} = A_{CLS:C} - (CLS_{M_{osm}} + CLS_{M_{ana}}) - T_{CLS:C} - CLS_{INT} \cdot G \tag{48}$$

$$\frac{dCHO_{INT}}{dt} = A_{CHO:C} - CHO_{M_{osm}} - T_{CHO:C} - CHO_{INT} \cdot G \tag{49}$$

$$\frac{dEPA_{INT}}{dt} = A_{EPA:C} - EPA_{M_{rep}} - T_{EPA:C} - EPA_{INT} \cdot G \tag{50}$$

$$\frac{dDHA_{INT}}{dt} = A_{DHA:C} - DHA_{M_{rep}} - T_{DHA:C} - DHA_{INT} \cdot G \tag{51}$$

$$\frac{dGLY_{INT}}{dt} = A_{GLY:C} - GLY_{M_{was}} - T_{GLY:C} - GLY_{INT} \cdot G \tag{52}$$

$$\frac{dGA_{INT}}{dt} = A_{GA:C} - GA_{M_{was}} - T_{GA:C} - GA_{INT} \cdot G \tag{53}$$

$$\frac{dCYS_{INT}}{dt} = A_{CYS:C} - CYS_{M_{was}} - T_{CYS:C} - CYS_{INT} \cdot G. \tag{54}$$

2.4. Quadrant metric

To illustrate *Daphnia's* overall fitness, we constructed a quadrant metric (QM), providing a look into the organism's somatic congeners (see Fig. 2). Specifically, the QM plots congener use efficiency (i.e., the fraction of assimilated congener mobilized for use) against nourishment (i.e., congener saturation), and split the plane into four segments. If a congener is located in quadrant 1 (defined by low use efficiency and low nourishment), the organism is likely *stressed* for this resource. This could be due to low dietary availability, low mobilization due to the hierarchical logic of our model, or a combination of the two. Congeners in quadrant 2 (defined by low use efficiency and high nourishment) indicate a *bottleneck*. High nourishment values may indicate high dietary availability, or simply reflect the low efficiency of use. The low use efficiency is likely resultant of our model's hierarchical logic, reflecting "upstream" congener depletion (e.g., low neurological saturation may lead to low energetic capacity, in turn causing low congener mobilization rates and a buildup of reserves). When congeners are predominantly in quadrant 3 (defined by high use efficiency and high nourishment), the organism is *functioning optimally*. If a congener is in this situation, it is abundantly available in the diet, and being put to use efficiently. Finally, if a congener lies in quadrant 4 (defined by high use efficiency and low nourishment), its use is optimized for the given scenario (i.e., an *opportunistic* response). That is, dietary availability is low, but a healthy fraction of the assimilated congener is put to use. If environmental conditions change, however, and dietary availability is stressed, the organism is likely in trouble.

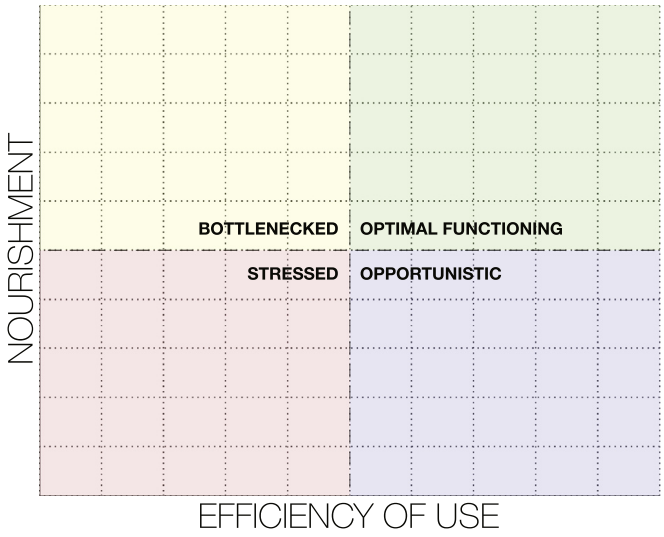


Fig. 2. Quadrant Metric (*QM*), illustrating the somatic status of modeled congeners. Congeners in abundant supply that are being used efficiently by the organism are considered *Optimally Functioning*, whereas efficient use with low somatic stores are *Opportunistic* responses. Congeners experiencing high somatic concentrations, but low efficiency of use are considered *Bottlenecked*, and congeners with low somatic concentrations and low use efficiency are labeled *Stressed*.

3. Results and discussion

3.1. Energetic partitioning

The fraction of energy allocated to osmoregulatory maintenance (E_{OSM}), is used to mobilize *P*, *CHO*, *FAT*, and *CLS* in varying quantities, for the upkeep of osmo-membranes. The bioenergetic hierarchy in our model first addresses maintenance costs, followed closely by waste management, and finally growth investments. Because the energetic partitioning (i.e., the fractions of energy allocated to maintenance, anabolism, and reproduction) is parameterized, various life history strategies can be tested. We stress that some of the following experiments are designed to stretch the model and demonstrate the range of dynamics outside of ecological and physiological norms. As such, the results are used to make inferences about our model's depiction of nature, and not discuss the quantitative implications.

A key assumption of our model is the 1:1 ratio of maintenance and waste management energetics. That is, for every unit of energy used for maintenance, another unit is used for recycling maintenance byproducts. Thus, the maximum energetic partition an individual can allocate to E_{OSM} is 50%. The remaining 50% in this case is allocated entirely to recycling, leaving no energetic resources for stoichiometric regulation or growth. Because of this tight relationship, we infer that individuals are faced with a tradeoff, placing a high priority on either maintenance, or growth. To illustrate the tradeoff, we scanned a continuous E_{OSM} trajectory from 0–40% (see Fig. 3a). Realized growth (*RG*; the fraction of maximum growth achieved) is 100% when E_{OSM} is set to zero, as all available energy is diverted to growth processes. As E_{OSM} increases, however, *RG* falls steadily because fewer energetic resources remain for growth. This linear decline continues until an abrupt shift, where *RG* falls to zero. We postulate that this breaking point (*BP*) occurs when the turnover module regulating supersaturated congener pools (i.e., pools with internal congener concentrations exceeding their optimum) switches on. When this occurs, the energetic ratio of maintenance to waste management falls below parity, as additional energetic units are required to expel internal substrates. The percentage of energy used for waste management (E_{WAS}) is also depicted against the continuum of E_{OSM}, and shows a steep rise at the *BP* coinciding with growth falling to zero. Individuals allocating large fractions of available energy to E_{OSM} are likely to experience limited growth. This in turn limits somatic congener utilization, and leads to supersaturation. At the onset of supersaturation, our model's logic triggers a negative feedback loop in the form of homeostatic congener turnover. In the current experiment, we stretched the model by further increasing E_{OSM} past the *BP*. Should an individual face supersaturation, we maintain our model's corrective dynamics of temporarily slowing/shutting down growth as a plausible response to an extremely unbalanced diet.

An indirect lesson from the previous experiment are the effects of an unbalanced diet on daphnid physiology. In testing the sensitivity of energetic partitioning, we observed how quickly sustained congener supersaturation compromised organism growth. The primary mechanism fueling congener saturation is ingestion. Because our model in its present form simulates the internal processes of individual animals and does not explicitly accommodate the interplay of the grazing rates with prey density or composition, we tested these aspects using parameter continuums (as in Fig. 3a). Specifically, we examined the effects of both E_{OSM} and nominal grazing (consumption) rate (*GRAZ*) on *RG* (see Fig. 3b). The inverse relation between *RG* and E_{OSM} was consistent with the previous experiment, but while *GRAZ* exerted minimal effects on *RG* dynamics, it did determine the point where a dramatic decline in the animal growth was experienced. The *GRAZ* at which *BP*s occurred decreased with an increasing E_{OSM}. A daphnid's resource consumption in our model can be counterbalanced by its energetic partitioning scheme (i.e., physiological priorities/strategy). That is, extremely high consumption levels are sustainable for individuals placing low emphasis on maintenance. This avoids a *BP* and a zero growth phase. Our model clearly postulates a conditional relationship between the capacity of animals to capitalize on food consumption and their energetic partitioning strategies, and pinpoints the delicate balance between consumption and effective utilization of internal resources as the key condition to maintain growth and avoid supersaturation.

Another facet of consumption is congener content in the grazed food, an aspect we tested using neuro- (Fig. 3c), energetic- (Fig. 3d), and physiological- (Fig. 3e) congeners (congeners not involved in neuro- or energetic-processes are referred to as physiological-congeners). These experiments were designed to stretch the system, and like the previous simulation, tested two independent variables against *RG*, while all others were held equal. Consistent with our previous results, high E_{OSM} values slowed animal growth, but became less of an issue as grazed neuro-congener concentration increased. That is, the organism was able to withstand extremely high E_{OSM} values when combined with high neuro-congener concentrations. Due to the hierarchical nature of our model, not all congeners are equal. Since neuro-congeners control how much energy is liberated for physiological processes, they exert dynamics fundamentally different from all “downstream” congeners. High neuro-congener concentrations liberate larger quantities of energy, which according to our results, can offset steep maintenance requirements. Neuro-congeners occupy the highest hierarchical status in our model, and all others respond in a markedly different manner to enrichment. The response of energetic-(i.e., mid-hierarchy), and physiological-(i.e., low-hierarchy) congeners to enrichment emphasizes the important balance between consumption and utilization, whereby enriched food sources can cripple growth in individuals by requiring higher energetic investments to waste management and maintenance of homeostasis. The lack of strict homeostasis in *Daphnia* is the central tenet of our framework, as illustrated by the differential internal congener concentrations and internal dynamics triggered by environmental variability. While outside the scope of the current study, it bears mention that temperature will also impart congener accumulation and expenditure stresses on *Daphnia* (Matthews and Mazumder, 2005). Because these experiments were designed to stretch two model parameters at a time, we have essentially illustrated our model's response to imbalances in dietary composition against energetic priorities. Food sources extremely rich in only one or a subset of resource(s) (with the

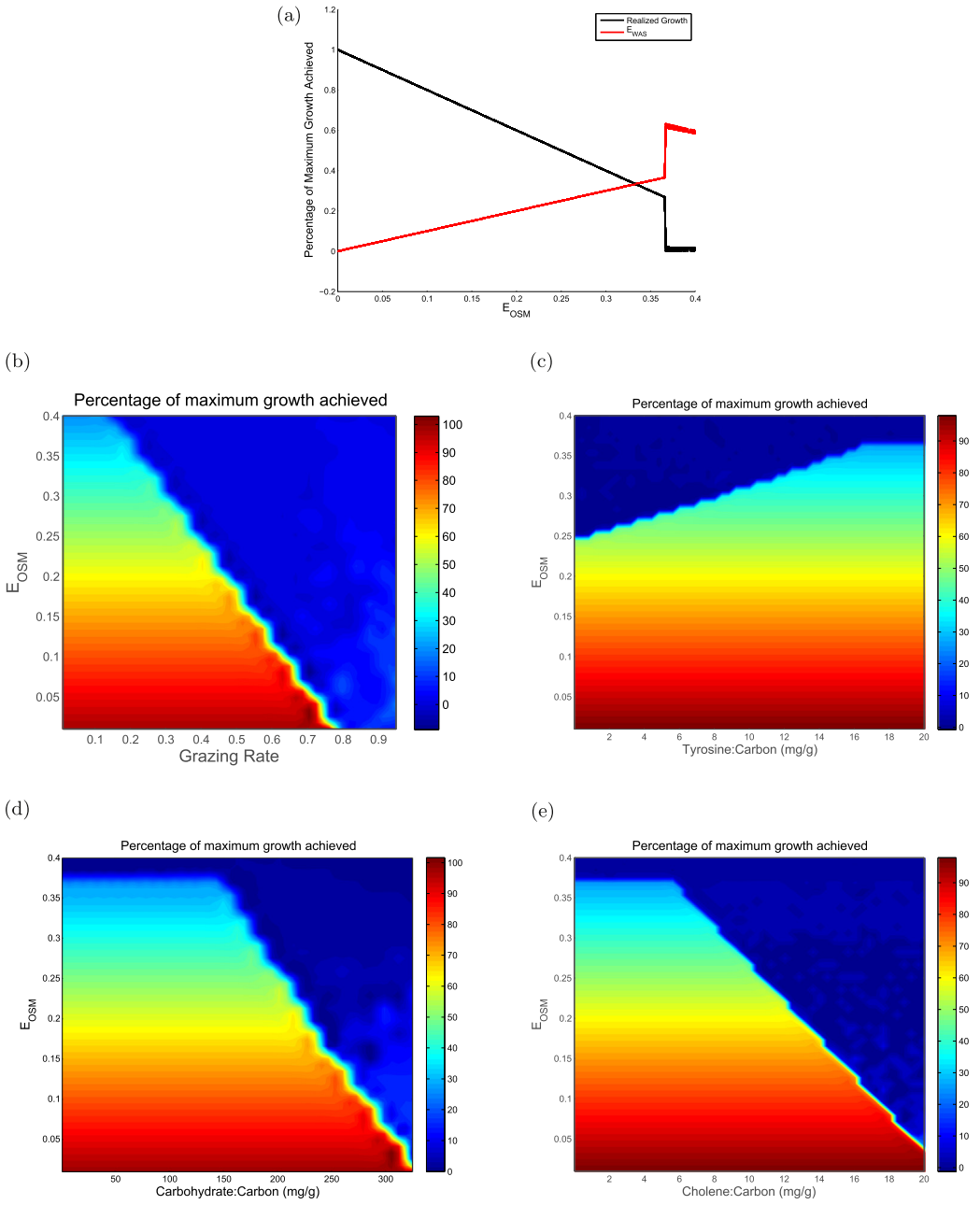


Fig. 3. Realized growth (*RG*) and energy requirements for waste management (E_{WAS}) against maintenance energetic requirements (E_{OSM}) (a). *RG* response to scanned E_{OSM} and grazing rate (*GRAZ*; b), neuro- (c), energetic- (d), and physiological- (e) congeners illustrate the unique influence of neuro-congeners, resultant of model hierarchy.

exception of neuro-congeners) can "overwhelm" our modeled daphnid, and compromise its somatic growth if energetic priorities, consumption, and utilization are not balanced.

The final set of exploratory analyses explicitly investigated the consumption–utilization balance. Thus far, both consumption rate and composition of ingested food have been linked to destabilization of an individual's energetic balance. We have suggested that this is likely due to a consumption–utilization mismatch, whereby the individual is accruing congeners faster than it can utilize them. Our previous experimental design deliberately forced this mismatch, but we now examine the emergence of a similar pattern against a variable utilization rate. Maximum growth rate (G_{MAX}) controls the maximum utilization rate of all congeners, and we hypothesize that increases in G_{MAX} may counter-balance (or at the very least buffer) somatic response to stress stemming from enrichment. We ran a series of four-dimensional sensitivity analyses, where *RG* was plotted against independent variables E_{OSM}, G_{MAX}, and congener concentration for neuro- (Fig. 4a), energetic- (Fig. 4b), and physiological- (Fig. 4c) congeners. Each plot was constructed by overlaying *RG* isosurfaces atop one another (see Movie 1 in Electronic Supplementary Material, referred to as ESM from here on out); each z-axis cross-sectional slice depicts *RG* in response to E_{OSM} and congener saturation, for a given G_{MAX} value (see Movie 2 in ESM). Having already discussed the importance of neuro-congeners to our modeled daphnid's physiology, the interplay between G_{MAX} and neuro-congener concentration was not surprising. In particular, low G_{MAX} values required pairing with very high neuro-congener concentrations in grazed food to avoid both *BP*s and crippled growth. Increased G_{MAX} alleviated the need for high neuro-congener saturations to avoid *BP*s. We posit that both of these combinations (i.e., high G_{MAX} and high neuro-congener saturation) increase congener utilization rates (through independent mechanisms), and thus minimize the likelihood of *BP* occurrence with enrichment. Conversely, energetic- and

Table 6
Daphnia parameterization for small and large individuals. Parameters not shown are assigned default values. Smaller individuals assumed to require less maintenance, with a focus on anabolic growth, while larger individuals have steeper maintenance requirements, and focus on reproductive growth. Grazing and growth rates are size dependent, and S_{FACTOR} is a somatic scaling factor controlling congener requirement. Minimum and optimum values for all congeners are scaled down to 40% in small individuals, and up to 150% in large individuals.

Parameter	Small	Large
E_{OSM}	0.05	0.35
E_{ANA}	0.85	0.15
E_{REP}	0.15	0.85
GRAZ	0.5	0.9
G_{MAX}	0.4	0.85
S_{FACTOR}	0.4	1.5

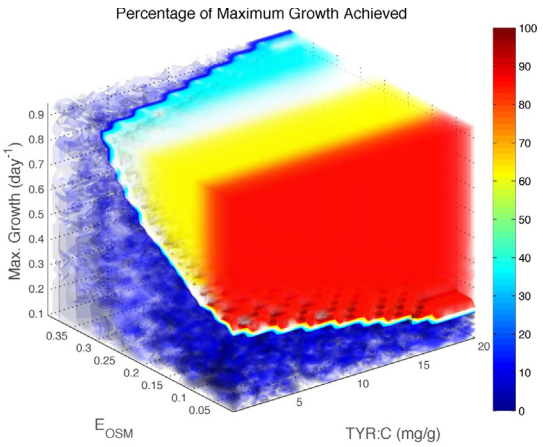
physiological-congeners required high G_{MAX} values to offset enrichment, effectively illustrating the balance between congener consumption and utilization. That is, individuals were able to sustain food with enriched congeners, as long as the maximum utilization rate was high.

3.2. Energetic partitioning summary

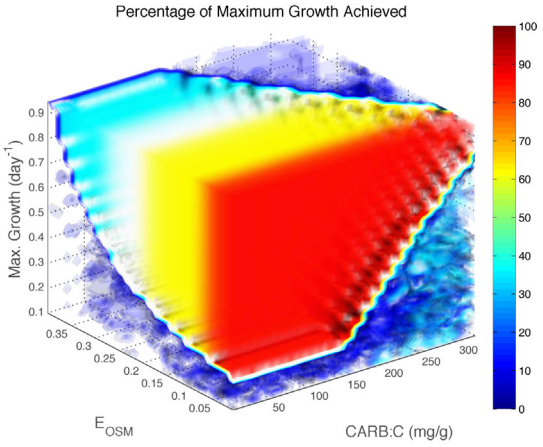
By exploring energetic partitioning schemes, we learned that organism response is dependent on a complex interplay among resource consumption, utilization, and energetic investment strategies. Daphnids modeled with high maintenance requirements tended to allocate fewer energetic resources to growth. This limited growth was directly related to the increased need to recycle maintenance by-products. Further, increased consumption may not always yield elevated animal growth. If intake is higher than what is required, congener supersaturation is reached, triggering a *BP*, where regulatory turnover is activated. In post-*BP* dynamics, the energetic ratio of maintenance:recycling is less than 1, generally resulting in near-zero growth. The onset of a *BP* can be minimized with a healthy consumption–utilization balance. That is, individuals with high growth rates fare better when facing extreme enrichment, as they can more readily utilize additional resources. Not all congeners are equal, however, and congener balance is very important. If the environment experiences enrichment across all congeners, our modeled daphnids are likely to prosper. By contrast, when only a subset of congeners is enriched, supersaturation can cascade across all pools. Energy diverted to address supersaturation, for example, limits the utilization of growth-related congeners, causing additional supersaturation and further increasing the energetic requirements of recycling. This positive feedback can readily destabilize the lower food web. The non-equality of resources stems from modeled logic and congener hierarchy, as our modeled daphnids respond uniquely to neuro-congener enrichment. Where unbalanced congener enrichment stressed an individual to the point of zero-growth, neuro-congeners were readily utilized (within the explored domain). Neuro-enrichment also had important impacts on subsequent processes, most notably energetics. Neuro-enrichment not only increased the saturation and utilization efficiency of neuro-congeners, but also increased the utilization efficiency of energetic-congeners.

3.3. QM benchmarks

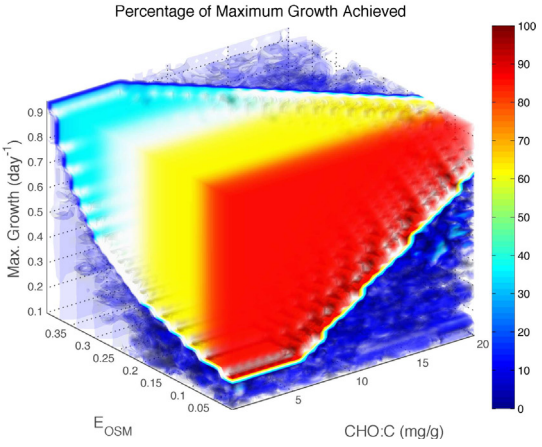
The *QM* was developed to provide an overview of our modeled daphnid's congener status. While the proposed paradigm has management implications (see Synthesis section), benchmarks are required to gauge and quantify physiological response. An example is testing how congener placement on the *QM* responds to shifting environmental conditions. Specifically, what is the *QM*'s response to congener enrichment scenarios similar to those tested earlier? A series of thematically controlled enrichmental simulations were run, gauging our daphnid's physiological response to neuro- (Movie 3 in ESM), energetic (Movie 4 in ESM), and physiological (Movie 5 in ESM) congener enrichment. In the case of neurological congeners, our previous finding of enrichment



(a) Tyrosine.



(b) Carbohydrate.



(c) Choline.

Fig. 4. Four dimensional plots, depicting *RG* against E_{OSM}, G_{MAX}, and neurological-(i.e., *TYR*), energetic-(i.e., *CARB*), and physiological-(i.e., *CHO*) congener concentrations.

leading to higher quantities of liberated energy held true. Specifically, as neuro-congeners were enriched, both the levels of somatic saturation and use efficiency increased, indicative of a neurological *bottleneck*. That is, the more neuro-congeners the organism ingested, the more it utilized them, and their placement on the *QM* migrated from *stressed* to

optimally functioning. As this bottleneck relaxed, more energetic congeners were mobilized, as indicated by the migration of *CARB*, *FAT*, and *PROT* from the *bottleneck* quadrant, to the *opportunistic* domain. Unlike the previous exercise, however, both neuro-congeners were manipulated (previously only *TYR* enrichment was explored), and a *BP* was experienced. Post-*BP* dynamics follow a positive feedback loop. On the *QM*, this is depicted as follows: one (or a subset of) congener(s) achieve supersaturation (congener reaches upper limit of *QM*'s y-axis); growth halts; downstream congener use is minimized; other congener pools reach supersaturation (congeners approach upper limit of y-axis on *QM*); use efficiency falls to 0% across all pools (congeners approach lower limit of *QM*'s x-axis). Similar to our *Energetic Partitioning* experiments, neurological enrichment dynamics were markedly different from those of energetic and physiological enrichment. Enriching energetic congener concentrations in the grazed food, for example, increased their somatic saturations only. Because neuro-congener concentrations were held constant, use efficiency did not change (solidifying the notion of a neuro-bottleneck). Downstream effects were minimal, as growth and waste management congeners experienced subtle increases in use efficiency. Physiological congeners (in this case, *EPA* and *DHA*), experienced similar increases in somatic saturation with enrichment, with no shifts in use efficiency, and no effects on any other congeners. *N* enrichment exhibited the same pattern (Movie 6 in ESM), but *P* was different. *P* enrichment not only increased somatic *P* saturation, but also decreased the efficiency of use (see Movie 7 in ESM). Growth and waste management congeners experienced shifts in use efficiency, as was the case with energetic enrichment, suggesting a slight increase in liberated energy with *P* enrichment. The fact that only neuro-congeners and *P* moved diagonally on the *QM*, while all others moved vertically reflects congener hierarchy. That is, enriching congeners that only control bottlenecked physiological processes will not benefit the organism (as illustrated on the *QM* with vertical movement). According to our framework, situations like this could easily result in supersaturation and a halt in growth. The latter finding once again emphasizes the importance of a balanced diet.

3.4. QM benchmark with seasonal variability in food quality

Using two algal characterizations (i.e., high and low quality; see Table 3), we created a seasonal algal composition by taking a weighted average of the two characterizations (see Fig. 5a). At the spring bloom, for example, the algal quality reflected 95% high quality, and 5% low quality, approximating diatom dominance. Conversely, at the peak of cyanobacteria dominance in mid-summer, the characterization was 5% high, and 95% low quality algae. We ran two individuals with vastly different physiological priorities against seasonality (see Table 6). *Individual A* was characterized with low maintenance requirements, low grazing and growth rates, low somatic quotas, and an emphasis on anabolic growth, while *Individual B* had higher maintenance requirements, high grazing and growth rates, comparatively higher somatic quotas, and an emphasis on reproductive growth. Physiological response of *Individuals A* and *B* are shown at the spring bloom and mid-summer (Fig. 5b–e). The general pattern observed indicated healthier individuals at the spring bloom, due to the high neuro- and physiological-congener availability, combined with high *Food Quality*. By contrast, both strategists experienced congener pool depletion, and reduced use efficiency in mid-summer, where *Food Quality* was very low. This was reflected in somatic congener concentrations, which in some instances were markedly lower. The *Food Quality* term represents algal morphological, toxicological, and ingestible traits, and can be seen as a resource bottleneck. That is, if a food source is very rich across all congeners, but has a very low *Food Quality* (e.g., low ingestibility due to cell wall thickening; see Van Donk et al., 1997), the grazing daphnid may not be able to assimilate a large fraction of the available resources (see Eq. (1)). While both strategists prospered and suffered simultaneously, the characteristics of each were very different. That is, *Individual A* tended to be more satisfied nutritionally at the spring bloom, and less stressed in mid-summer than *Individual B*. This can be attributed to a number of factors, starting with somatic quota size. Since *Individual A* was characterized with somatic quotas smaller than *Individual B*, it can be seen as more opportunistic. Small quota requirements led to quicker nutritional satisfaction, as indicated by *bottlenecked* or *optimally functioning* somatic congeners. Conversely, *Individual B*'s congener pools were consistently found in the *stressed*, or *opportunistic* regions. Both individuals were parameterized with balanced grazing and growth rates, avoiding the input/output mismatch observed in earlier model stretching experiments.

4. Synthesis and future perspectives

Identifying optimal model structure requires selecting an appropriate level of detail to portray the problem at hand, the ramifications of which can ultimately determine the credibility of the inference drawn (Brooks and Tobias, 1996). Most analytical approaches in theoretical ecology use models developed to capture high level topological features (e.g., species richness, number of trophic levels, linkage density) (Garcia-Domingo and Saldana, 2007). In this context, Seth (2002) offered a new perspective by tying together two distinct themes in his thesis: the relation of behavioral properties to environmental structure, and the distinction between behavioral and mechanistic levels of description. We continued this line of thought by modeling a daphnid's physiological processes and priorities (i.e., behavioral description), and built out to organism level dynamics (i.e., mechanistic description). We modeled daphnid response to environmental nutrition (i.e., the quality and quantity of available food) by quantifying its physiological potential (i.e., nutritional status). This potential drove the simulated physiological processes, which will in turn modulate organism interactions with the environment. We posit that animal behavior and physiological state follow molecular dynamics, which indirectly govern higher level functions (e.g., resource acquisition, reproduction, energy expenditure). For this reason, our blending of ecology with daphnid physiology is a new take on an old management problem.

Because organisms quickly respond to toxic stress at the metabolic level (i.e., environmental metabolomics), the characterization of an organism's low molecular weight metabolite content can characterize organism responses to natural, anthropogenic, and/or biotic stressors (Lankadurai et al., 2013). Following short-term exposure to sub-lethal metal concentrations, Nagato et al. (2013) observed significant changes in *Daphnia magna* metabolite composition. Specifically, amino acid concentrations fell in response to exposure, suggesting either slowed production, or expedited depletion to ward off toxicity. Metal exposure can also alter neurotransmitter synthesis and impair organism energetics (Nagato et al., 2013). This level of observational detail sheds light on the mechanisms underlying an organism's phenotype, and deepens our understanding of causal responses. Another example is that of cyanobacteria, which are often axiomatically treated as a poor food source for *Daphnia* spp. (Müller-Navarra et al., 2004). Various hypotheses strive to address why this is the case (e.g., stoichiometric imbalance, HUFA mismatch, mechanical interference, toxicity), but definitive evidence has yet to be published. In the era of metabolomics, *Daphnia* spp.'s internal response to inferior quality algae can be observed, quantified, and compared. In particular, our analysis showed that zooplankton's ability to overcome unfavorable compositional changes in algal assemblages can vary significantly depending on somatic requirements (i.e., consumer stress is proportional to the mismatch between consumer congener requirements and producer composition), and capacity to adapt congener utilization rates. These types of comparisons will not only build ecophysiological theory, but can also significantly boost modeling efforts.

We advocate for a management approach where metabolomic data is used to parameterize our ecophysiology model. The present model can potentially be used as an ecosystem health metric, in addition to a forecasting tool for scenario-based evaluation. A dynamic model of a keystone species can alert policy makers to potential ecosystem

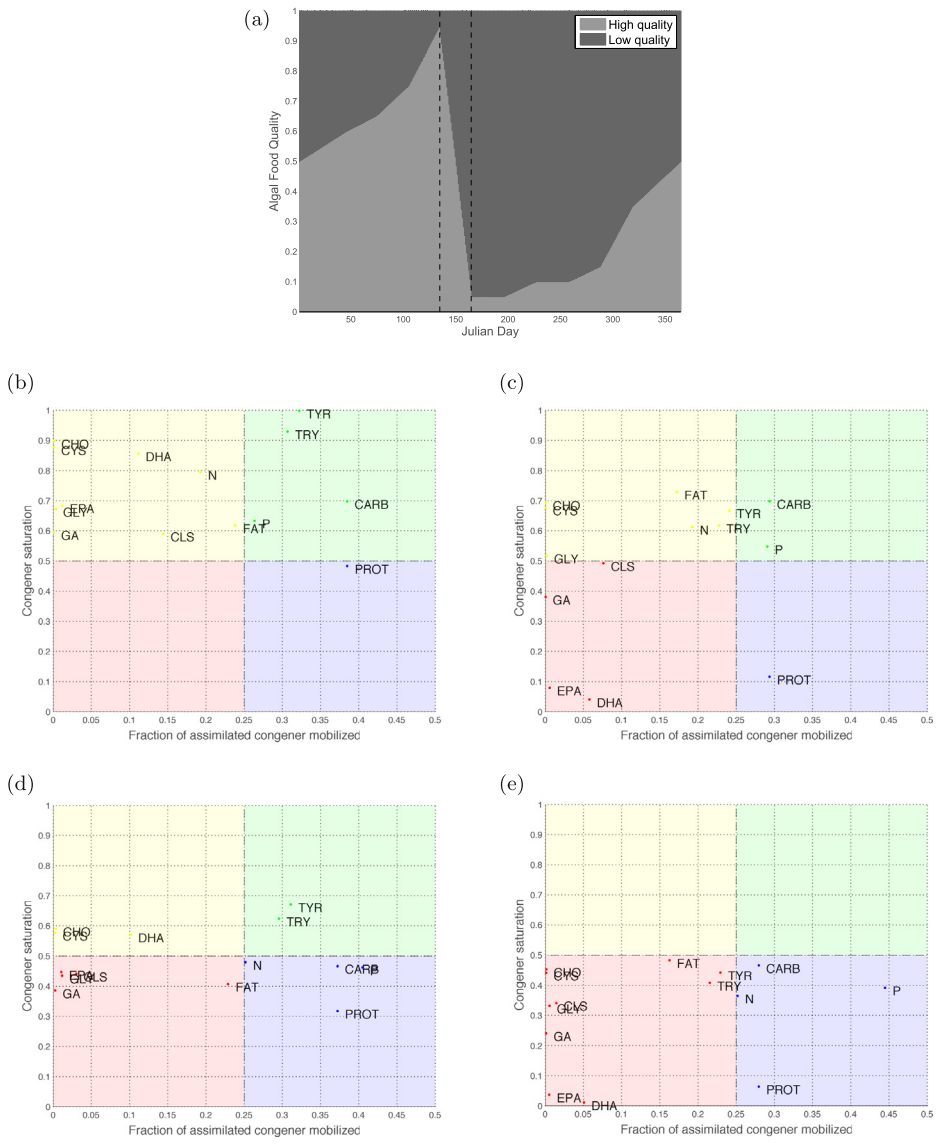


Fig. 5. Seasonal algal composition continuum illustrating quality of food available for *Daphnia* spp. (a). Dashed lines represent scans conducted at the spring bloom (b,d), and mid-summer (c,e), for Individual A (characterized with low grazing and growth rates, low quota requirements, low maintenance requirements, and a strong emphasis on anabolic growth; panels b & c) and Individual B (characterized with high grazing and growth rates, high quota and maintenance requirements, and a strong emphasis on reproductive growth; panels d & e).

sensitivities and stresses before they become apparent. Breakthroughs in the field of metabolomics open up a new realm of scientific investigation, and the quality of data produced from these breakthroughs allows us to refine traditional management models with more robust parameter specifications and formulations (Simpson et al., 2012; Lankadurai et al., 2012). The simplicity of static zooplankton characterizations (i.e., rigid organismal stoichiometry) is convenient when fitting models to environmental conditions delineated by calibration datasets, but can ultimately introduce artifacts in other aspects of ecosystem functioning (see discussion about the role of zooplankton in recycling non-limiting nutrients by Arhonditsis and Brett (2005) versus Perhar et al. (2012c)). Stoichiometric theory suggests that animals are able to maintain relatively constant somatic elemental compositions, regardless of environmental conditions (Sterner and Elser, 2002). We argue, however, that a zooplankter modeled with this framework will be unrealistically compromised when somatic requirements are not fulfilled by environmental resource availability. In other words, a model of zooplankton growth based on rigid and non-adaptive requirements is likely to overestimate die-off events and abrupt shifts in system dynamics. This is not likely the case in nature, as organisms are generally adapted to natural variability in their environments (Ferrão-Filho et al., 2007). In the present modeling framework, the assumption of rigid somatic stoichiometry is relaxed and organism dynamics emerge from a set of biological rules, encompassing bioenergetics, balancing of physiological process, and evolutionary strategies.

There are various criteria (paraphrased in Table 2) to consider when creating an ecosystem indicator (Doren et al., 2009). We chose to project our modeled daphnid's health metric onto a two-dimensional Cartesian plane divided into four quadrants. This allowed for similarly behaving metabolites to be grouped together. Determining which metabolic congeners share dynamical patterns is important, as it can shed light on which physiological functions are disproportionately affected by the prevailing conditions, and thus effectively offer an early warning signal. Incorporating a calibrated version of our proposed daphnid model into a food web context (outside the scope of the current study) could be used to establish times-series based threshold patterns. This daphnid-centric food web model would build patterns from processes, and could conceivably offer new insights into microscopic to macroscopic pattern progression. For example, explicitly accounting for time would add another dimension to the *QM*, whereby the rate of

change in congener accumulation and use could be used to indicate the severeness of an early warning sign. In the present form of the *QM*, congeners can occupy each of the four quadrants in the domain studied. Thus, the individual can be simultaneously classified as *optimally functioning*, *opportunistic*, *bottlenecked*, and *stressed*, across its various congeners. Once any congener crosses into the *stressed* quadrant, there is a cause for concern. Our hierarchical physiology suggests, however, that not all stress-related concerns are equal. A neurologically stressed individual, for example, poses a more serious concern than one lacking "luxury" congeners (i.e., downstream congeners associated with anabolic and reproductive processes). This is due to the cascading effects upstream congeners can have on seemingly non-related physiological processes. Our model configuration also postulates that an imbalanced diet rendering a small subset of congeners above saturation, may trigger a feedback loop of congener accrual, followed by a decline in growth. This could conceivably signal an impending collapse/shift at the food web level. Thus, we stress that one of the main challenges is to define rules on how animals cope with near-starvation or resource over-enrichment conditions.

Scaling from an individual to an entire population, we intend to deploy an agent-based approach, where population heterogeneity is intrinsically built into our framework. The two-pronged framework (organism dynamics → ecosystem variability) presented herein is a first attempt to build a management-oriented model founded upon metabolome data (see Fig. 6). However, the modeling of physiological processes at one organizational level and the subsequent consideration of the control exerted on the dynamics of different trophic levels entail considerable uncertainty. Both facets of our framework are prone to multiple interpretations and mathematical descriptions, and thus may lead to a Type I error. That is, "false positives" or "false alarms", predicting an erroneously high likelihood of an undesirable shift in the ecosystem state. It may also result in a Type II model error, or "false negatives". These arise when the predictions are unjustifiably comforting and fail to identify lurking threats to the ecosystem's integrity. The credibility of our proposed risk assessment methodology will be determined by our understanding of the underlying science. As a first approximation, we have made assumptions that are based on data availability (e.g., congeners explicitly considered and their respective fates), while others are hypothesis driven (e.g., physiological hierarchy in the congener utilization). Revisiting these assumptions as more data becomes available can strengthen our understanding of daphnid physiology. In addition, we acknowledge the inherent complexity in allometrically characterizing *Daphnia* and quantifying their health against a wide range of stressors. Revising physiological assumptions, such as minimum and optimum somatic congener concentrations, turnover rates, and mobilization rates will also likely impact metric performance. Introducing additional causal linkages may also enhance model realism. For example, the amino acids cysteine, glutamic acid, and glycine are required for indirectly disposing maintenance by-products and supersaturated congeners before somatic and reproductive growth can occur; this can be revisited. Another major undertaking will be the explicit consideration of the toxicity patterns induced by the exposure to (sub)lethal contaminant levels. Finally, the increased complexity of our metabolite-driven daphnid ecophysiological model invites a rigorous assessment of the underlying uncertainty and the amount of information needed to ensure reasonable parameter identifiability (Arhonditsis et al., 2007; McCulloch et al., 2013)

Being our first attempt to cross daphnid physiology with lower food web dynamics, we have done our best to maintain simplicity. Gaps in our fundamental understanding of metabolite interactions are compounded by lack of data, and as such, we acknowledge that our qualitative results may come with considerable margins of error. One source of uncertainty arises from our choice of congeners. In the current state of the model, not all congeners are essential (i.e., some can be synthesized *de novo* and/or bioconverted from other congeners). This obfuscates congener fate, as non-essential congener depletion may reflect heightened downstream congener production, or a reduced availability in upstream congeners (Nagato et al., 2013). The current state of our model does not account for congener biotransformations, or their associated energetic requirements. The level of eco-physiology presented here has the potential to open a new window into our understanding of freshwater ecosystem response to anthropogenic disturbances. The *QM* classification scheme combined with time-explicit dynamics (i.e., time-series analysis) has the potential to ascend the three ecological indicator levels outlined by Rapport and Hilden (2013): conceptual (i.e., theory building), legitimizing (i.e., using information in non-decision making context), and instrumental (i.e., used in policy-making decisions). We expect our knowledge of daphnid

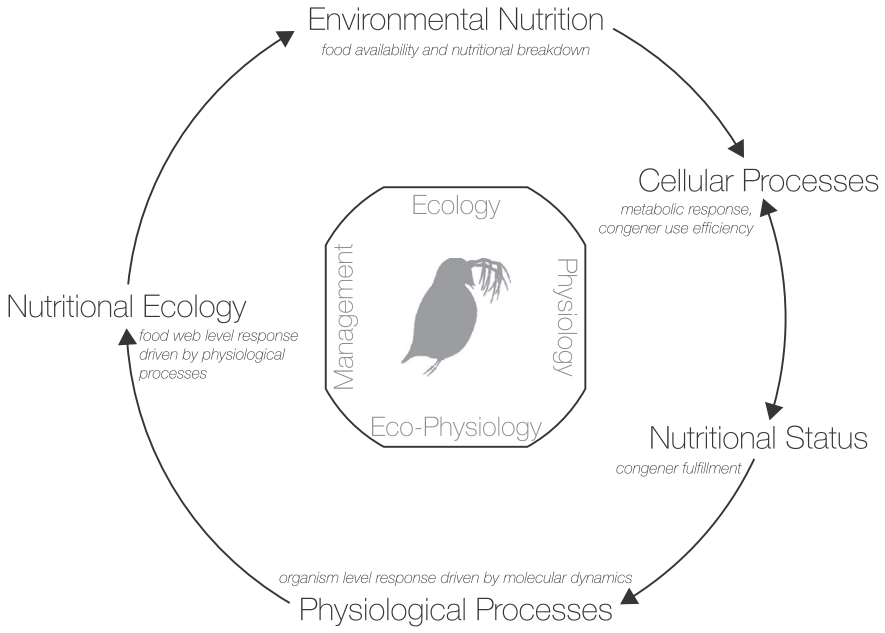


Fig. 6. Model structure depicting the combination of ecological and physiological factors to produce a management tool.

physiology to change as more metabolite data become available, and anticipate a considerable back and forth dynamic, where laboratory findings push modeling endeavors, and vice-versa (Flynn, 2006).

Please view the accompanying Electronic Supplementary Material, containing additional Tables, Figures, and Animations referred to in the manuscript. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecoinf.2015.09.002.

Acknowledgments

This project has received funding support from the Krembil Foundation. Additional funding for Gurbir Perhar was provided by a MITACS Elevate Postdoctoral Fellowship. We would like to thank Dong Kyun Kim, Felicity Ni, Edward Nagato, and Brian Lankadurai for their editorial comments, and Ranbir Perhar for his philosophical input with an earlier variant of the model.

References

Aalto, S.L., Kaski, O., Salonen, K., Pulkkinen, K., 2013. Response of algae, bacteria, *Daphnia* and natural parasite fauna of *Daphnia* to nutrient enrichment in mesocosms. Hydrobiologia 715, 5–18.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., Walter, P., 2002. Dna replication, repair, and recombination. Chapter *in* Molecular Biology of the Cell. Garland Science.

Aliev, A.E., Harris, K.D.M., 2004. Probing hydrogen bonding in solids using solid state NMR spectroscopy. Struct. Bond. 108, 1–53.

Altshuler, I., Demiri, B., Xu, S., Constantin, A., Yan, N.D., Cristescu, M.E., 2011. An integrated multi-disciplinary approach for studying multiple stressors in freshwater ecosystems: *Daphnia* as a model organism. Integr. Comp. Biol. 51, 623–633.

Anderson, T.R., Hessen, D.O., Elser, J.J., 2005. Metabolic stoichiometry and the fate of excess carbon and nutrients in consumers. Am. Nat. 165, 1–15.

Ang, C.Y., 1953. Activation energies and diffusion coefficients of oxygen and nitrogen in niobium and tantalum. Acta Metall. 1, 123–125.

Arhonditsis, G.B., Brett, M.T., 2005. Eutrophication model for Lake Washington (USA) Part II — model calibration and system dynamics analysis. Ecol. Model. 187, 179–200.

Arhonditsis, G.B., Qian, S.S., Stow, C.A., Lamon, E.C., Reckhow, K.H., 2007. Eutrophication risk assessment using Bayesian calibration of process-based models: application to a mesotrophic lake. Ecol. Model. 208, 215–229.

Biesinger, K.E., Christensen, G.M., 1972. Effects of various metals on survival, growth, reproduction, and metabolism of *Daphnia magna*. J. Fish. Res. Board Can. 29, 1691–1700.

Billiard, S.M., Meyer, J.N., Wassenberg, D.M., Hodson, P.V., Di Giulo, R.T., 2008. Nonadditive effects of PAHs on early vertebrate development: mechanisms and implications for risk assessment. Toxicol. Sci. 105, 5–23.

Brooks, R.J., Tobias, A.M., 1996. Choosing the best model: level of detail, complexity, and model performance. Math. Comput. Model. 24, 1–14.

Bundy, J.G., Davey, M.P., Viant, M.R., 2009. Environmental metabolomics: a critical review and future perspectives. Metabolomics 5, 3–21.

Cantrell, K.B., Walker, T.H., 2009. Influence of temperature on growth and peak oil biosynthesis in a carbon-limited medium by *Pythium irregulare*. J. Am. Oil Chem. Soc. 86, 791–797.

Colbourne, J.K., Pfrender, M.E., Gilbert, D., Thomas, W.K., Tucker, A., Oakley, T.H., Tokishita, S., 2011. The ecoresponsive genome of *Daphnia pulex*. Science 331, 555–561.

Doren, R.F., Trexler, J.C., Gottlieb, A.D., Harwell, M.C., 2009. Ecological indicators for system-wide assessment of the greater everglades ecosystem restoration program. Ecol. Indic. 9, S2–S16.

Droop, M.R., 1968. Vitamin B12 and marine ecology. IV. The kinetics of uptake, growth and inhibition in *Monochrysis Lutheri*. J. Mar. Biol. Assoc. U. K. 48, 689–733.

Ferrão-Filho, A.D.S., Tessier, A.J., DeMott, W.R., 2007. Sensitivity of herbivorous zooplankton to phosphorus-deficient diets: testing stoichiometric theory and the growth rate hypothesis. Limnol. Oceanogr. 52, 407–415.

Filippov, A., Oradd, G., Lindblom, G., 2003. The effect of cholesterol on the lateral diffusion of the phospholipids in oriented bilayers. Biophys. J. 84, 3079–3086.

Flynn, K.J., 2006. Dynamics of P-binding forms in sediments of a mesotrophic hard-water lake: insights from non-steady state reactive-transport modeling, sensitivity and identifiability analysis. J. Plankton Res. 28, 873–875.

Food and Agriculture Organization of the United Nations, 2014. URL http://www.fao.org/docrep/006/y5022e/y5022e04.htm.

Fussmann, G.F., Heber, G., 2002. Food web complexity and chaotic population dynamics. Ecol. Lett. 5, 394–401.

Gajbhiye, S.N., 2002. Zooplankton — study methods, importance and significant observations*in*. In: Quadros, G. (Ed.), Proceedings of the National Seminar on Creeks, Estuaries and Mangroves, pp. 21–27.

Garcia-Domingo, J.L., Saldana, J., 2007. Food-web complexity emerging from ecological dynamics on adaptive networks. J. Theor. Biol. 247, 819–826.

Garibaldi, A., Turner, N., 2004. Cultural keystone species: implications for ecological conservation and restoration. Ecol. Soc. 9, 1–18.

Griffiths, W., 2007. Metabolomics, Metabonomics and Metabolite Profiling. Royal Society of Chemistry, Cambridge.

Guisande, C., Gliwicz, Z.M., 1992. Egg size and clutch size in 2 *Daphnia* species grown at different food levels. J. Plankton Res. 14, 997–1007.

Heffner, L.J., Schust, D.J., 2010. The Reproductive System at A Glance. 3rd edition. John Wiley and Sons, New York.

Hietala, J., Reinikainen, M., Walls, M., 1995. Variation in life history response of *Daphnia* to toxic *Microcystis aeruginosa*. J. Plankton Res. 17, 2307–2318.

Jager, T., Martin, B.T., Zimmer, E.I., 2013. DEBkiss or the quest for the simplest generic model of animal life history. J. Theor. Biol. 328, 9–18.

Jansen, M., Vergauwen, L., Vandenbrouck, T., Knapen, D., Dom, N., Spanier, K.I., Cielen, A., Meester, L.D., 2013. Gene expression profiling of three different stressors in the water flea *Daphnia magna*. Ecotoxicology 22, 900–914.

Kooijman, S.A.L.M., 2001. Quantitative aspects of metabolic organization: a discussion of concepts. Philos. Trans. R. Soc. B 356, 331–349.

Kurbatova, S.A., 2005. Response of microcosm zooplankton to acidification. Biol. Bull. 32, 85–92.

Lampert, W., 1987. Feeding and nutrition in *Daphnia*. Mem. Ist. Ital. Idrobiol. 45, 143–192.

Lankadurai, B.P., Simpson, A.J., Simpson, M.J., 2012. Elucidation of the toxic mode of action of perfluorooctanoic acid and perfluorooctane sulfonate to the earthworm *Eisenia fetida* after sub-lethal exposure using ^{1}H NMR-based metabolomics. Environ. Chem. 9, 495–501.

Lankadurai, B.P., Nagato, E.G., Simpson, M.J., 2013. Environmental metabolomics: an emerging approach to study organism responses to environmental stressors. Environ. Rev. 21, 180–205.

Libralato, S., Christensen, V., Pauly, D., 2006. A method for identifying keystone species in food web models. Ecol. Model. 195, 153–171.

Loureiro, C., Pereira, J.L., Pedrosa, M.A., Goncalves, F., Castro, B.B., 2013. Competitive outcome of *Daphnia–Simocephalus* experimental microcosms: Salinity versus priority effects. PLoS ONE 8.

Martin-Creuzburg, D., Wacker, A., von Elert, E., 2005. Life history consequences of sterol availability in the aquatic keystone species *Daphnia*. Oecologia 144, 362–372.

Matthews, B., Mazumder, A., 2005. Temporal variation in body composition (c:N) helps explain seasonal patterns of zooplankton delta c-13. Freshw. Biol. 50, 502–515.

McCulloch, J., Gudimov, A., Arhonditsis, G.B., Chesnyuk, A., Kittrich, M., 2013. Dynamics of P-binding forms in sediments of a mesotrophic hard-water lake: insights from non-steady state reactive-transport modeling, sensitivity and identifiability analysis. Chem. Geol. 354, 216–232.

Mendonca, S., Cardoso, G., Caraca, J., 2015. Some notes on the strategic strength of weak signal analysis. Technical Report. Lisbon Internet and Networks International Research Programme.

Micholson, J.K., Lindon, J.C., 2008. Metabonomics. Nature 455, 1054–1056.

Miner, B.E., Meester, L.D., Pfrender, M.E., Lampert, W., Hairsten Jr., N.G., 2012. Linking genes to communities and ecosystems: *Daphnia* as an ecogenomic model. Proc. R. Soc. B 279, 1873–1882.

Müller-Navarra, D.C., Brett, M.T., Park, S., Chandra, S., Ballantyne, A.P., Zorita, E., 2004. Unsaturated fatty acid content in seston and tropho-dynamic coupling in lakes. Nature 427, 69–72.

Nagato, E.G., D'eon, J.C., Lankadurai, B.P., Poirer, D.G., Reiner, E.J., Simpson, A.J., Simpson, M.J., 2013. ^{1}H NMR-based metabolomics investigation of *Daphnia magna* responses to sub-lethal exposure to arsenic, copper and lithium. Chemosphere 93, 331–337.

Nicholson, J.K., Lindon, J.C., Holmes, E., 1999. Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica 29, 1181–1189.

Nurnsten, H., 2005. The Maillard Reaction: Chemistry, Biochemistry and Implications. The Royal Society of Chemistry.

Paine, R.T., 1969. A note on trophic complexity and community stability. Am. Nat. 103, 91–93.

Paine, R.T., 1995. A conversation on refining the concept of keystone species. Conserv. Biol. 9, 962–964.

Patterson, M.J., Findlay, D.L., Salki, A.G., Hendzel, L.L., Hesslein, R.H., 2002. The effects of *Daphnia* on nutrient stoichiometry and filamentous cyanobacteria: a mesocosm experiment in aeutrophic lake. Freshw. Biol. 47, 1217–1233.

Perhar, G., Arhonditsis, G.B., Brett, M.T., 2012a. Modeling the role of highly unsaturated fatty acids in planktonic food web processes: a mechanistic approach. Environ. Rev. 20, 155–172.

Perhar, G., Arhonditsis, G.B., Brett, M.T., 2012b. Modeling the role of highly unsaturated fatty acids in planktonic food web processes: sensitivity analysis and examination of contemporary hypotheses. Ecol. Inf. 13, 77–98.

Perhar, G., Arhonditsis, G.B., Brett, M.T., 2012c. Modelling crustacean highly unsaturated fatty acids in Lake Washington: a mechanistic approach to physiology in a eutrophication model. Ecol. Model. 258, 101–155.

Persson, J., Brett, M.T., Vrede, T., Ravet, J.L., 2007. Food quantity and quality regulation of trophic transfer between primary producers and a keystone grazer (Daphnia) in pelagic freshwater food webs. Oikos 116, 1152–1163.

Plagemann, P., 1971. Choline metabolism and membrane formation in rat hepatoma cells grown in suspension culture. III. Biosynthesis of phospholipids from radioactive precursors in rabbit renal cortex slices. Biochemistry 5, 423–435.

Rapport, D.J., Hilden, H., 2013. An evolving role for ecological indicators: from documenting ecological conditions to monitoring drivers and policy responses. Ecol. Indic. 28, 10–15.

Rosenzweig, M., 1971. The paradox of enrichment. Science 171, 385–387.

Rothhaupt, K.O., 1997. Grazing and nutrient influences on *Daphnia* and *Eudiaptomus* on phytoplankton in laboratory microsystems. J. Plankton Res. 19, 125–139.

Royle, J.A., Dorazio, R.M., 2008. Hierarchical Modeling and Inference in Ecology. Academic Press.

Scheffer, M., Carpenter, S.R., 2003. Catastrophic regime shifts in ecosystems: linking theory to observation. Trends Ecol. Evol. 18, 648–656.

Scheffer, M., Carpenter, S., Foley, J.A., Folke, C., Walker, B., 2001. Catastrophic shifts in ecosystems. Nature 413, 591–596.
Scheffer, M., Carpenter, S.R., Lenton, T.M., Bascompte, J., Brock, W., Dakos, V., vandeKoppel, J., Leemput, I.A., Levin, S.A., Van Nes, E.H., Pascual, M., Vandermeer, J., 2012. Anticipating critical transitions. Science 338, 344–348.
Seda, J., Petrusek, A., 2011. *Daphnia* as model organism in limnology and aquatic biology: introductory remarks. J. Limnol. 70, 337–344.
Seth, A.K., 2002. Agent-based modelling and the environmental complexity thesis. Proceedings of the Seventh International Conference on Simulation of Adaptive Behavior. MIT Press, pp. 13–24.
Simpson, A.J., Simpson, M.J., Soong, R., 2012. Nuclear magnetic resonance spectroscopy and its key role in environmental research. Environ. Sci. Technol. 46, 11488–11496.
Sjoberg, S., 1980. Zooplankton feeding and queueing theory. Ecol. Model. 10, 215–225.
Sorf, M., Davidson, T.A., Brucet, S., Menezes, R.F., Sondergaard, M., Lauridsen, T.L., Landkildehus, F., Liboriussen, L., Jeppesen, E., 2015. Zooplankton response to climate warming: a mesocosm experiment at contrasting temperatures and nutrient levels. Hydrobiologia 742, 185–203.
Sperfeld, E., Wacker, A., 2009. Effects of temperature and dietary sterol availability on growth and cholesterol allocation of the aquatic keystone species *Daphnia*. J. Exp. Biol. 212, 3051–3059.
Sterner, R.W., Elser, J.J., 2002. Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere. Princeton University Press, New Jersey.
Straile, D., Adrian, R., Schindler, D.E., 2012. Uniform temperature dependency in the phenology of a keystone herbivore in lakes of the northern hemisphere. PLoS ONE 7, e45497.
Torrent, J., 1991. Activation energy of the slow reaction between phosphate and goethites of different morphology. Aust. J. Soil Res. 29 (1), 69–74.
Van Donk, E., Lurling, M., Hessen, D.O., Lokhorst, G.M., 1997. Altered cell wall morphology in nutrient-deficient phytoplankton and its impact on grazers. Limnol. Oceanogr. 42, 357–364.
Van Doorslaer, W., Stoks, R., Duvivier, C., Bednarska, A., Meester, L.D., 2009. Population dynamics determine genetic adaptation to temperature in *Daphnia*. Evolution 63, 1867–1878.
Wacker, A., Martin-Creuzburg, D., 2007. Allocation of essential lipids in *Daphnia magna* during exposure to poor food quality. Funct. Ecol. 21, 738–747.
Wagner, A., Benndorf, J., 2007. Climate-driven warming during spring destabilises a *Daphnia* population: a mechanistic food web approach. Oecologia 151, 351–364.
Wang, H., Dunning, K., Elser, J.J., Kuang, Y., 2009. Daphnia species invasion, competitive exclusion, and chaotic coexistence. Discret. Contin. Dyn. Syst. Ser. B 12, 481–493.
Whitfield, P.D., German, A.J., Noble, P.M., 2004. Metabolomics: an emerging postegenomic tool for nutrition. Br. J. Nutr. 92, 549–555.
Wu, J., Chen, Z., Dovichi, N.J., 2000. Reaction rate, activation energy, and detection limit for the reaction of 5-furoylquinoline-3-carboxaldehyde with neurotransmitters in artificial cerebrospinal fluid. J. Chromatogr. B Biomed. Sci. Appl. 741, 85–88.
Yoshii, H., Furuta, T., Georges, C., Mizoguchi, S., Soottitantawat, A., Linko, P., 2006. Kinetic analysis of autoxidative docosahexaenoate triglyceride in the presence of rosemary extract with oxygen sensor. In: Teale, M.C. (Ed.)Omega 3 Fatty Acid Research vol. 290. Nova Science Pub Inc., pp. 109–118.

TOWARDS THE DEVELOPMENT OF AN EARLY WARNING SYSTEM FOR ECOSYSTEM HEALTH: A METABOLITE-DRIVEN DAPHNIA ECOPHYSIOLOGICAL MODEL

ELECTRONIC SUPPLEMENTARY MATERIAL

Gurbir Perhar and George B. Arhonditsis

Ecological Modelling Laboratory, University of Toronto. 1265 Military Trail, Scarborough, Ontario, M1C-1A4, Canada

August 8, 2014

Table 1: Congener availability and morphological food quality of high and low quality algae. Experiments not specifying algal parameterization defaulted to high quality. Congener units are $\mu gmgC^{-1}$, FQ is unitless. The primary differences between high and low quality algae are neurocongener concentrations, energetic congener concentrations, HUFA concentrations, and morphological features.

PARAMETER	HIGH	LOW
TRY	19.60	14.70
TYR	23.38	17.53
$CARB$	99.40	112.0
FAT	70.82	101.17
$PROT$	89.92	25.15
CHO	5.80	5.80
CHL	11.96	11.96
EPA	12.07	1.81
DHA	5.80	0.078
CYS	10.15	10.15
GLY	2.36	2.36
GA	17.73	14.73
P	3.25	3.25
N	51.45	51.45
FQ	0.9	0.025

Table 2: Energetic yields of carbohydrates, fat, and proteins, taken from the Food and Agriculture Organization of the United Nations' website (2014).

PARAMETER	VALUE	UNIT
$CARB_{YIELD}$	4	$\frac{kcal}{g}$
FAT_{YIELD}	9	$\frac{kcal}{g}$
$PROT_{YIELD}$	4	$\frac{kcal}{g}$

Table 3: Congener activation energies and sources.

CONGENER	VALUE	UNIT	SOURCE
CHL	$55-65$	$\frac{kJ}{mol}$	Filippov et al. (2003)
CHO	16	$\frac{kcal}{mol}$	Plagemann (1971)
EPA	36.5	$\frac{mol}{kJ}$	Cantrell and Walker (2009)
DHA	30	$\frac{kJ}{mol}$	Yoshii et al. (2006)
CYS	$29.4-32.2$	$\frac{kcal}{mol}$	Nurnsten (2005)
GA	34.4	$\frac{kJ}{mol}$	Wu et al. (2000)
GLY	$24-30$	$\frac{kcal}{mol}$	Aliev and Harris (2004)
N	36.8	$\frac{kcal}{mol}$	Ang (1953)
P	46	$\frac{kJ}{mol}$	Carmona et al. (1994)

Table 4: *Daphnia* parameterization for small and large individuals. Parameters not shown are assigned default values. Smaller individuals assumed to require less maintenance, with a focus on anabolic growth, while larger individuals have steeper maintenance requirements, and focus on reproductive growth. Grazing and growth rates are size dependent, and S_{FACTOR} is a somatic scaling factor controlling congener requirement. Minimum and optimum values for all congeners are scaled down to 40% in small individuals, and up to 150% in large individuals.

PARAMETER	SMALL	LARGE
E_{OSM}	0.05	0.35
E_{ANA}	0.85	0.15
E_{REP}	0.15	0.85
$GRAZ$	0.5	0.9
G_{MAX}	0.4	0.85
S_{FACTOR}	0.4	1.5

References

Food and Agriculture Organization of the United Nations. 2014; `http://www.fao.org/docrep/006/y5022e/y5022e04.htm`.

Filippov, A.; Oradd, G.; Lindblom, G. *Biophysical Journal* **2003**, *84*, 3079–86.

Plagemann, P. *Biochemistry* **1971**, *5*, 423–35.

Cantrell, K. B.; Walker, T. H. *Journal of the American Oil Chemists' Society* **2009**, *86*, 791–7.

Yoshii, H.; Furuta, T.; Georges, C.; Mizoguchi, S.; Soottitantawat, A.; Linko, P. In *Omega 3 Fatty Acid Research*; Teale, M. C., Ed.; Nova Science Pub Inc., 2006; Vol. 290; Chapter 5, pp 109–18.

Nurnsten, H. *The Maillard Reaction: Chemistry, Biochemistry and Implications*; The Royal Society of Chemistry, 2005.

Wu, J.; Chen, Z.; Dovichi, N. J. *J Chromatogr B Biomed Sci Appl* **2000**, *741*, 85–8.

Aliev, A. E.; Harris, K. D. M. *Structure and Bonding* **2004**, *108*, 1–53.

Ang, C. Y. *Acta Metallurgica* **1953**, *1*, 123–5.

Carmona, D.; Ferrer, J.; Lamata, M. P.; Oro, L. A. *Journal of Organometallic Chemistry* **1994**, *470*, 271–4.