

Prediction of *Daphnia* Production along a Trophic Gradient

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ABSTRACT: To predict *Daphnia* secondary productivity along a trophic gradient indexed as total phosphorus (TP) concentration, we estimated energy transfer efficiencies from food quality for *Daphnia* such as eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) content. Eleven flow-through *Daphnia magna* growth experiments were conducted with seston from 9 lakes, ponds and river waters. Primary productivities were estimated from food supply rates in the flow-through experiments, producing energy transfer efficiencies from seston to *D. magna*. We found DHA content was the best predictor of energy transfer efficiencies among the essential fatty acids. An asymptotic saturation model explained 79.6% of the variability in energy transfer efficiencies. Based on empirical data in this study and empirical models from literature, we predict that *Daphnia* productivity would peak in mesotrophic systems by decreasing food quality and increasing food quantity along trophic gradient.

Key words: *Daphnia*, DHA, Fatty acids, Flow-through experiment, Secondary productivity, TP, Trophic gradient

INTRODUCTION

Energy flow at the primary producer and herbivore interface is influenced by both quantity and quality of foods available for zooplankton in freshwater pelagic ecosystems (Hessen and Faafeng 2000, Müller-Navarra et al. 2004). In general, filter feeders such as daphnids would consume higher amounts of phytoplankton in more productive waters (Smith 1979). However, food quality for daphnids, measured through highly unsaturated fatty acid content such as eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) content, has been reported to decrease as water becomes more eutrophic because cyanobacteria usually dominate more-productive freshwater ecosystems (Müller-Navarra et al. 2004).

Since energy transfer efficiency at the producer-herbivore interface can be defined as the ratio of secondary productivity to primary productivity (Hilbricht-Ilkowska 1977), secondary productivities may be predicted with given primary productivities and energy transfer efficiencies. Seston food quality in terms of phosphorus (P) (Hessen and Faafeng 2000) and essential fatty acids (Park et al. 2003, Müller-Navarra et al. 2004) have been proposed as important factors determining energy transfer efficiencies at the seston and filter-feeding zooplankton interface in freshwater ecosystems. Recent empirical studies suggested that molar C:P ratio above 300 would limit growth of *Daphnia* (Brett et al. 2000, Park et al. 2002).

In this study, we attempted to predict *Daphnia* secondary pro-

ductivity along a trophic gradient indexed as total phosphorus (TP) concentration by estimating energy transfer efficiencies from food quality indicators for *Daphnia* such as EPA or DHA content.

MATERIALS AND METHODS

For a series of flow-through *Daphnia magna* growth experiments, we collected water from various water bodies including artificial reservoirs, ponds, and rivers in California, USA, between January and August 2002 (Table 1). Among 9 waters, Suisun Slough at Suisun City and Lagoon Reservoir were sampled twice providing waters for flow-through growth experiments. In our flow-through experiments, water was screened with 30 μm mesh to provide seston with edible sizes for *D. magna*. In the flow-through experiments, eight neonates of 3-day-old *D. magna*, birth time synchronized within 12 hr, were fed with seston freshly collected on each experiment day for 4 days without light. Each flow-through experiment had 2~3 seston sources (waters from different sites and cultured *Scenedesmus acutus*) which fed three replicated 250 mL animal chambers (8 animals per chamber). These chambers received a constant food supply from stirred reservoirs (2 L Erlenmeyer flasks) with a multichannel peristaltic pump (1.44 L day⁻¹) in a 20°C temperature controlled room.

Production efficiency was used as energy transfer efficiency in this study according to Hilbricht-Ilkowska (1977):

$$\text{Production efficiency: } P_h / P_p \quad (1)$$

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where P_h indicates productivity at the herbivore level and P_p denotes productivity at the primary producer level.

We postulated that primary production (P_p) could be estimated from supplied amounts of seston to *D. magna* if there was no additional algal production during a flow-through experiment as follows:

$$P_p = \text{carbon content in seston} \times \text{food supply rate} \\ [\mu\text{g C chamber}^{-1} \text{ day}^{-1}] \quad (2)$$

To ensure no additional algal production, we conducted our flow-through experiments under no-light conditions. Since *Daphnia* feeding tended to saturate above 700 mg C L⁻¹ (unpublished data), we diluted seston to a carbon content of approximately 0.5 mg C L⁻¹ using filtered lake water whenever carbon content in seston was estimated at greater than 0.5 mg C L⁻¹. Too much carbon content would result in an underestimate of energy transfer efficiencies. Actual supplied carbon content ranged between 154 ~ 605 μg C L⁻¹ except for Stonegate pond with 1,150 μg C L⁻¹ (Table 1). Secondary production of *D. magna* was measured as daily average increase of carbon in animals using weight and carbon content differences between the start and the end of each flow-through growth experiment.

Fatty acids, carbon content and total and particular phosphorus in seston were measured for seston foods collected on each experiment day for flow-through growth experiments. Each fatty acid was identified by the retention time from a separate run of a 37 fatty acids methyl ester mix (Supelco). We used 10 mL of heptacosanoic acid (21:0; 1 mg mL⁻¹) as an internal standard, which was added onto the freeze-dried filter immediately prior to the extraction process to determine fatty acid concentrations.

Samples were analyzed with a gas chromatograph (HP6890) with

a programmable temperature vaporizer (PTV) and a flame ionization detector (FID). Fatty acids were quantified by calculating the area ratios of a sample compared to the internal standard. Response factors for the single fatty acid standards were obtained from comparisons of quantitative fatty acid standards and the internal standard. The differences between estimated fatty acid concentrations from the internal standard and quantitative standards were <5%. For seston carbon analyses, 100 mL of water was filtered onto precombusted 13 mm Whatman GF/C filters. Filters were dried at 60°C for 2 days, placed in tin foils and analyzed for carbon and nitrogen using an in-line elemental analyzer (PDZ Europa Scientific, ANCA-GSL). Total phosphorus (TP) and soluble reactive phosphorus (SRP) were determined to estimate particulate phosphorus content by subtracting SRP from TP; both were measured by colorimetric methods (Eaton et al. 2005). All elemental ratios are reported as molar ratios.

Along a trophic gradient, primary productivity is well known to increase linearly as would total phosphorus concentration (TP) (Smith 1979):

$$\text{Primary Productivity} = 10.4 \times TP - 79 [\mu\text{g C L}^{-1} \text{ day}^{-1}] \quad (3)$$

Also, a recent study showed a pattern of decreasing DHA along a TP gradient (Müller-Navarra et al. 2004):

$$\text{Log}_n \text{ DHA} = -1.11 \times \text{Log}_n \text{ TP} + 3.42 [\mu\text{g FA L}^{-1}] \quad (4)$$

We selected an asymptotic saturation model (Müller-Navarra 1995, Park et al. 2003) for DHA and energy transfer efficiencies.

$$\text{Efficiency} = a_0 \times (1 - e^{a_1 \times \text{DHA} + a_2}) [\%] \quad (5)$$

Table 1. Characteristics of seston used in the flow-through experiments. Numerals in parentheses are standard deviations of averages left.

Site	<i>n</i>	PC [μg L ⁻¹]	Diluted Carbon [μg L ⁻¹]	TP [μg L ⁻¹]	EPA [μg mg C ⁻¹]	DHA [μg mg C ⁻¹]	Molar C:P
Dr. Jameson's Pond	1	2285	500	21	21.8	3.8	483
Lake Solano	1	184	184	25	2.9	0.1	28
Lake Berryessa	1	154	154	27	3.0	1.7	21
Cameron Park Lake	1	421	421	43	3.0	0.9	69
Lake Natoma	1	551	500	45	5.5	2.8	79
Sacramento River at Rio Vista	1	677	265	108	3.6	0.2	37
Stonegate Pond	1	4934	1150	168	0.7	0.1	199
Suisun Slough at Suisun City	2	1623(71)	605(96)	424(55)	2.5(0.9)	1.2(0.9)	74
Lagoon Reservoir	2	17620(4912)	337(32)	2049(424)	0.8(0.7)	0.7(0.2)	88

n: number of replicates, PC: particulate carbon concentration, TP: total phosphorus, C:P: carbon to phosphorus ratios.

where *Efficiency* is the energy transfer efficiency between seston and *D. magna*, a_0 is the maximum energy transfer efficiency (i.e. the asymptote), DHA is DHA content ($\mu\text{g FA mg C}^{-1}$), a_1 and a_2 are parameters determining slope and intercept of the model.

Combining primary productivity pattern and DHA gradient along TP gradient (equation 3 and equation 4) and energy transfer efficiency measurement (equation 5), we attempted to predict *D. magna* production along an eutrophication (TP) gradient.

RESULTS

We investigated two seston quality indices: essential fatty acids and phosphorus content (carbon to phosphorus ratios; molar C:P ratios). We did not observe severe phosphorus limitation (molar C:P ratio > 300) except for Dr. Jameson's Pond (Table 1). Since energy transfer efficiency for Dr. Jameson's Pond was among the highest

measured, we shifted focus to essential fatty acid content as an important factor in determining energy transfer efficiencies in this study.

First, we explored correlation patterns between energy transfer efficiencies and essential fatty acids such as α -linolenic acid (ALA), octadecatetraenoic acid (OCT), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (Fig. 1). Energy transfer efficiencies between seston and *D. magna* showed positive correlations with essential fatty acid contents except for ALA, which showed unimodal patterns. Since a recent study on essential fatty acid content patterns on TP gradient showed no clear pattern in ALA content in seston (Müller-Navarra et al. 2004), we considered OCT, EPA, and DHA as factors in energy transfer efficiencies. We examined possible combinations of three independent variables in linear models explaining energy transfer efficiencies (Table 2). Including two independent variables such as OCT and DHA or EPA and DHA showed higher coefficients of determination (r^2) than DHA only.

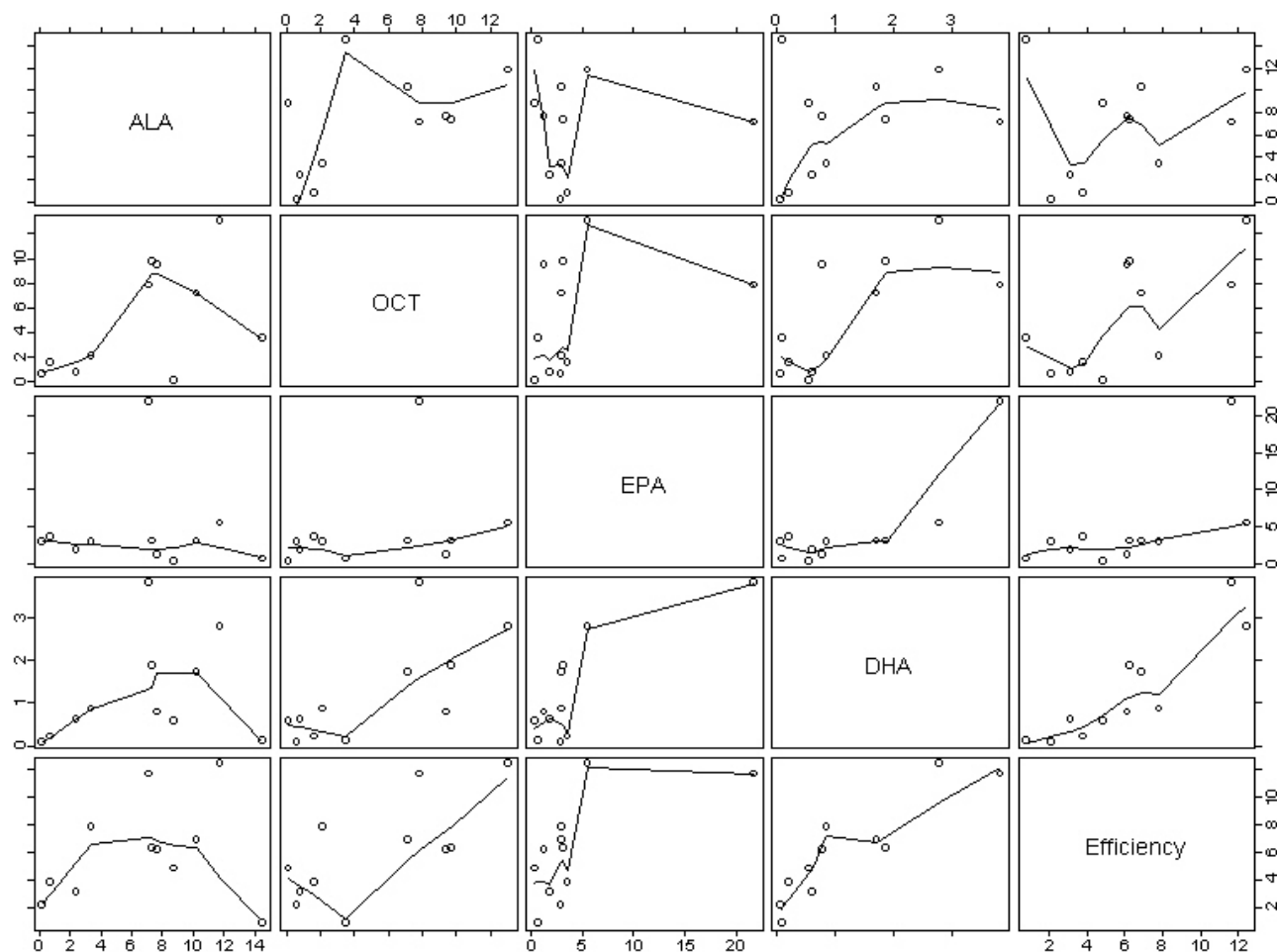


Fig. 1. Relationships among each fatty acid content in seston and energy transfer efficiencies (Efficiency) measured or estimated in this study. X and Y axes for fatty acids have unites of $\mu\text{g FA mg C}^{-1}$ while X and Y axis for efficiency has no unit. Lines were drawn by lowest function in S-Plus. ALA: α -linolenic acid, OCT: octadecatetraenoic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid.

Table 2. Coefficients of determination (r^2), adjusted coefficients of determination (adjusted r^2), and Akaike's Information Criterion (AIC) for each linear model using fatty acid content explaining energy transfer efficiencies between seston and *D. magna*

Model	r^2	Adjusted r^2	AIC
Efficiency ~ OCT	50.1	44.6	57.1
Efficiency ~ EPA	40.9	34.3	59.0
Efficiency ~ DHA	80.5	78.3	46.8
Efficiency ~ OCT + EPA	69.3	61.7	53.7
Efficiency ~ OCT + DHA	81.3	76.6	48.3
Efficiency ~ EPA + DHA	82.5	78.1	47.6
Efficiency ~ OCT + EPA + DHA	74.9	75.0	50.0

OCT: octadecatetraenoic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid.

However, criteria with penalties on numbers of independent variables such as adjusted r^2 and Akaike's Information Criterion (AIC) revealed that a model with DHA as the only independent variable explained energy transfer efficiencies best (Table 2).

The simplest model, that of DHA content effect on energy transfer efficiency, would be a linear model. However, it is unlikely one would observe continuously increasing energy transfer efficiencies because other factors would limit zooplankton growth beyond a certain level of DHA. DHA showed a saturating pattern with energy transfer efficiencies; EPA showed a similar saturating pattern even clearer than DHA (Fig. 1).

From nonlinear fitting, we obtained our model parameters as follows:

$$\text{Efficiency} = 12.5096 \times (1 - e^{-0.939 \times \text{DHA} + 0.1}) \quad (6)$$

This model explained 79.6% of the variation in energy transfer efficiency using DHA content ($\mu\text{g FA L}^{-1}$) (Fig. 2). As DHA content decreases, energy transfer efficiencies are predicted to decrease exponentially with TP increase. Decreasing efficiencies and increasing primary productivity produce a secondary productivity pattern in which *D. magna* would have the highest productivity in mesotrophic lakes around $40 \mu\text{g TP L}^{-1}$ (Fig. 3).

DISCUSSION

Our results show that *Daphnia* production would be highest in mesotrophic systems due to the combination of increasing primary productivities (food quantity) and decreasing energy transfer effi-

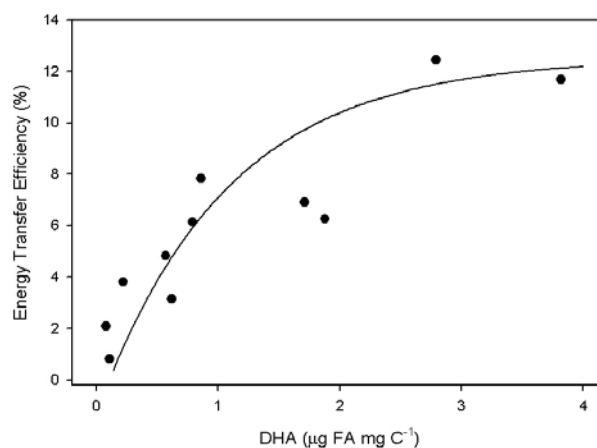


Fig. 2. Relationships between DHA content and energy transfer efficiencies from seston to *D. magna* estimated in flow-through experiments. The fitted equation was obtained using Microsoft Excel Solver function maximizing r^2 and zeroing bias.

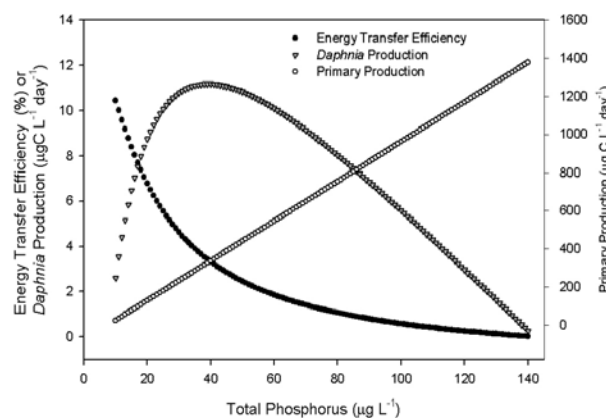


Fig. 3. Predicted patterns of energy transfer efficiencies (●), primary productivity (○) and *D. magna* productivity (▽) from total phosphorus concentration.

ciencies (food quality). This kind of unimodal pattern of secondary production has been well reported since the 1970s (Hilbricht-Ilkowska 1977, Jeppesen et al. 2003). Our present study also supports a recent model study that predicts *Daphnia* growth peaks in mesotrophic lakes due to low food quantity in oligotrophic lakes and low EPA in eutrophic lakes (Persson et al. 2007). The model study predicts that maximum *Daphnia* growth rates occur around $10 \sim 25 \mu\text{g TP L}^{-1}$ or $25 \sim 40 \mu\text{g TP L}^{-1}$ depending on equations used (Persson et al. 2007). Their TP ranges for maximum *Daphnia* growth appear very close to our value (around $40 \mu\text{g TP L}^{-1}$).

A recent study of 466 arctic and temperate lakes reported that *Daphnia* relative biomass peaked at $90 \sim 150 \mu\text{g TP L}^{-1}$ (Jeppesen et al. 2003). The report explained the decreasing percentage of *Daphnia* at high TP as the result of an increasing proportion of

planktivorous fish (Jeppesen et al. 2003). Our study attempted to predict *Daphnia* production rather than *Daphnia* biomass or relative abundance (Jeppesen et al. 2003). Since we did not include mortality factors such as zooplanktivorous fish predation, our results cannot predict *Daphnia* biomass along a trophic gradient. However, it is interesting to note from our interpretation that a lower intensity of fish predation would not rapidly release *Daphnia* biomass in eutrophic or hypereutrophic ecosystems due to low quality foods. A recent study simulated several scenarios of combinations of phytoplankton food quality and fish predation. It showed little increase in zooplankton biomass with lower fish predation compared to higher fish predation cases when food quality is low (Danielsdottir et al. 2007). In our view, less energy transfer efficiency due to cyanobacteria dominance is at least an important determinant for low *Daphnia* production and thus low biomass (Müller-Navarra et al. 2004). More data would be necessary to clarify when and where planktivorous fish predation or cyanobacteria dominance become more important for *Daphnia* biomass in eutrophic systems.

Another interesting aspect to consider is lower *Daphnia* production predicted in spite of high energy transfer efficiencies with lower TP values in our study. However, the P limitation hypothesis would expect low energy transfer efficiencies due to high C:P ratios in systems with lower TP (Hessen 2006, Danielsdottir et al. 2007). Further examination should resolve food qualities and thus energy transfer efficiencies of oligotrophic seston for daphnids.

A series of recent studies on essential fatty acids focused on EPA (Müller-Navarra 1995, Müller-Navarra et al. 2000, Müller-Navarra et al. 2004) or ω 3-polyunsaturated fatty acid (Park et al. 2002) rather than DHA. However, in our present study EPA saturated at a much lower value than DHA (Fig. 1), which may have led to DHA explaining trophic transfer efficiencies better than did EPA.

Our results are based on laboratory flow-through growth experiments with *D. magna* in which primary productivity was estimated from food supply rates. We think that our estimations of primary productivity may have resulted in some uncertainties in obtaining energy transfer efficiencies. It would be necessary to measure both primary and secondary productivity simultaneously in natural systems, in conjunction with food quality indices in terms of essential fatty acid content and P content, to fully support the results of this study.

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