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# Coupling of dietary phosphorus and growth across diverse fish taxa: a meta-analysis of experimental aquaculture studies

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*Abstract*. Imbalances in phosphorus (P) intake relative to demand negatively affect animal growth, but their consequences are less understood for vertebrates, in which bone represents a significant and potentially flexible pool of P. Flexibility in body-P content could buffer vertebrates from the effects of imbalances between P intake and demand, reducing the likelihood of a sharp stoichiometric "knife-edge" in the relationship between growth rate and diet-P level. We conducted a meta-analysis of published aquaculture experiments that tested effects of diet %P on fish growth rate (49 studies, 28 species) and body-P content (27 of the studies in the main data set, 20 species). Our meta-analysis revealed significant P limitation of growth, as well as significant negative effects of excess P on growth rate. Diet-P thresholds for these effects occurred at ecologically relevant levels (optimal diet-P of 1.2% ± 0.45%, mean ± SD, under experimental conditions of high ration). Finally, the analysis also suggested a pattern of relatively shallow relationships between growth rate and diet-P level, coupled with surprisingly flexible body-P content in fishes. This result is consistent with fish using flexible body-P content (presumably mediated through bone P) to buffer imbalances between P intake and demand. Together, our results provide evidence for a relatively "dull" stoichiometric "knife-edge" in fishes, driven in part by flexible body-P content.

*Key words: diet; ecological stoichiometry; elemental homeostasis; fishes; growth rate hypothesis; phosphorus limitation; stoichiometric knife-edge; vertebrates.*

## Introduction

The unique function played by phosphorus (P) in the structure of several key biomolecules makes it essential to all life (Sterner and Elser 2002). Its vital role in the diet of metazoans is amply illustrated by the consequences of mismatches in its supply relative to demands for growth. Diets deficient in P relative to carbon (C) are associated with lowered growth and other vital rates, as shown in many classic studies, including convincing demonstrations using the microcrustacean *Daphnia* (e.g., Urabe et al. 1997). In contrast, adverse effects of high diet-P intake have only been recognized more recently. Although the physiological mechanism is not well understood, deleterious effects of a high-P diet have been shown in several consumers from diverse taxonomic groups (Boersma and Elser 2006). Animals have therefore been described as negotiating a stoichiometric "knife-edge," either side of which lie the negative consequences of under- and oversupply of P in their diet (Elser et al. 2012).

Much stoichiometric theory on the coupling of dietary P and growth is based on studies of invertebrates and is encapsulated by the "growth rate hypothesis" (GRH; Elser et al. 1996, 2003). The GRH stresses the central importance of P for the production of ribosomal ribonucleic acid (rRNA), which is ~9% P and constitutes the cellular machinery for protein assembly, a prerequisite for growth. The GRH has found broad support across diverse taxa (Sterner and Elser 2002). Although the importance of rRNA for P demand transcends phylogeny, application of the GRH to vertebrates is complicated by growth of bone, which represents a second, significant P sink (Gillooly et al. 2005). If bone also represents a flexible P pool, it could buffer vertebrates from imbalances between P demand and intake, lessening the effects of a stoichiometric "knife-edge." Unfortunately, and despite the importance of vertebrates in most food webs, the relationships between dietary P intake, body-P content, and vertebrate growth have received far less attention from ecologists. However, a large body of data does exist, produced by dozens of experiments largely published in the aquaculture literature. Although others have pointed out the existence of these studies (Boersma and Elser 2006, Boersma et al. 2008), their synthesis has not been attempted until now.

Although not designed to test ecological theory, aquaculture experiments represent a rich source of information for testing hypotheses related to vertebrate stoichiometry. These studies have involved a taxonom-

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ically diverse group of species and are both explicit and highly replicated tests for the effects of dietary P on growth (and often many other variables) that control for other factors such as diet composition and food quantity. They also focus on a key group: fish are by far the largest class of vertebrates. Finally, fish exhibit a staggering diversity of body plans, life histories, and trophic strategies (Helfman et al. 2009), making them a useful model for other vertebrate groups.

Here, we use the combined results of published aquaculture experiments to examine relationships among dietary P content, relative P demand, and growth across diverse fish taxa. We surveyed studies that manipulated dietary P content while controlling for other diet constituents. We used the resulting data set to ask several questions about the role of P supply in determining fish growth rates. First, what is the evidence for P limitation of growth under conditions of ample food supply? Second, what is the incidence of inhibition of fish growth by high-P diets? Third, how flexible are fish with respect to body-P content, and how is their P content related to the coupling of dietary P and growth? Last, do relationships among diet-P, growth rate, and body-P content vary across trophic groups or habitat types? Our analysis reveals the costs of P imbalance for fish growth, and sheds light on the role of flexible body-P content in ameliorating these negative effects.

## Methods

### *Data collection and pretreatment*

We searched the literature for experimental studies that tested for effects of dietary P content on fish growth. Relevant studies were initially identified using combinations of the following search terms in the ISI Web of Knowledge database (now the Thomson Reuters Web of Science) and Google Scholar: "fish," "growth," "phosphorus," "diet," "dietary," and "body content" (*online databases*).[5,6] Once these studies were collated, literature cited within them was examined for additional studies not retrieved using either database. Diet-P content and growth data were extracted from tables and text within each study; where only presented in figures, we used GraphClick to capture data (*available online*).[7] Studies used in the analysis were restricted to those published from 1 January 1970 to 15 February 2012. Only studies that reported data for total diet-P (as opposed to assimilable P) and those without confounding variables (e.g., other treatments, such as dietary calcium content) were considered. Lastly, we only selected studies with the potential to assess peaks in growth: experiments with fewer than four treatment levels of diet-P content were excluded from the initial data set. We also excluded a small number of studies because of difficulties in translation.

Once the initial set of candidate studies was compiled, we screened studies for a relatively complete, unimodal growth response to large ranges in diet-P (i.e., studies in which a peak growth rate could be characterized). Studies with monotonic responses may have used a narrow diet P range or inadequate diet P levels, not sufficient for fully characterizing the growth–dietary P relationship. To screen for such studies, we sequentially implemented four criteria. First, we tested each growth–dietary P relationship for monotonicity using a bootstrap approach (Murtaugh 2003) and the 'Iso' package in the R statistical environment (R Development Core Team 2013, Turner 2013). Relationships deemed monotonic by the test were excluded. We further screened the remaining studies by excluding those with a coefficient of determination for the quadratic fit of $<0.5$, and those with negative linear or positive quadratic coefficients in the quadratic model (i.e., poorly fit or U-shaped relationships).

In each study, we converted data to calculate specific growth rates (SGR, $d^{-1}$) in each treatment of diet-P level:

$$\mathrm{SGR} = \log_e(M_\mathrm{f}/M_\mathrm{i})/t$$

where $M_\mathrm{f}$ is mean final wet mass, $M_\mathrm{i}$ is the mean initial wet mass, and $t$ is the duration of the feeding trial in days. When possible, we also extracted whole-body P content (at the conclusion of the experiment) for each treatment of diet-P level. In rare cases where body-P content was reported as %P wet mass and % dry mass was not also given, we assumed 75% body mass as water (Hartman and Brandt 1995). Mean body mass for each study was estimated as the geometric mean of the initial and final body masses in the treatment with the highest growth rate. Each experimental water temperature (or midpoint if a range) was noted to the nearest 0.1°C. To obtain additional information, such as trophic group (herbivore, predator or omnivore) and habitat (marine or freshwater), for each species, we used FishBase (*available online*).[8] Rearing conditions of individual studies dictated habitat type for diadromous species. All continuous variables, except water temperature, were ln-transformed to meet statistical assumptions of normality.

### *Growth responses to diet-P content*

The relationship between SGR and diet-P content ($\mathrm{diet_P}$) was fit to a quadratic equation

$$\mathrm{SGR} = \beta_\mathrm{I} + \beta_\mathrm{L}\mathrm{diet_P} + \beta_\mathrm{Q}\mathrm{diet_P^2}$$

where $\beta_\mathrm{I}$ is the intercept, $\beta_\mathrm{L}$ is the linear coefficient, and $\beta_\mathrm{Q}$ is the quadratic coefficient. The linear ($\beta_\mathrm{L}$) and quadratic ($\beta_\mathrm{Q}$) coefficients were used as metrics of the

[5] http://thomsonreuters.com/thomson-reuters-web-of-science/
[6] http://www.scholar.google.com
[7] http://www.arizona-software.ch
[8] http://www.fishbase.org

severity of P limitation and P inhibition, respectively (see *Mixed-effects meta-analysis*). We analytically solved for the vertex of the quadratic relationship using the linear and quadratic coefficients from the quadratic model to calculate the peak SGR as well as the optimal diet %P (OP), the diet %P at which peak SGR occurred. To estimate 95% confidence intervals for OP, we first used nonlinear least squares to fit the model:

$$\mathrm{SGR} = \beta_Q(\mathrm{diet_P} - \mathrm{OP})^2 + \mathrm{peak\ SGR}.$$

Then, for each study, we obtained a bootstrap distribution of model coefficients by resampling with replacement and fitting the nonlinear model ($n = 5000$, "nlsBoot" in the R package nlstools; Baty and Delignette-Muller 2013). The lower and upper 95% confidence limits for OP were the 2.5th and 97.5th percentiles of the bootstrap distribution of the model coefficient OP.

### *Stoichiometric homeostasis and body-P content at peak SGR*

The homeostasis parameter $H$ describes how body-P content responds to variation in diet-P content (Sterner and Elser 2002). We calculated $H$ in each reporting study by regressing the natural log of body-P content against the natural log of diet-P content. $H$ is calculated as the inverse slope of this regression line. To avoid values of infinity, we used $1/H$ (i.e., the slope of the regression line), which increases with stoichiometric flexibility. Finally, we also calculated the body-P content at peak SGR by solving the log-linear relationship between body-P and diet-P for the body-P content at optimum diet-P.

### *Testing phylogenetic signal in response variables*

Phylogenetic signal in all response variables entered into the model selection was assessed using Blomberg's $K$ (Blomberg et al. 2003) calculated using a time-calibrated phylogeny (see Appendix A for methods) and "phylosig" in the "phytools" package in R (Revell 2012). Evidence for phylogenetic signal in response variables was weak (see *Results*), so we proceeded with the following meta-analysis without phylogenetic correction.

### *Mixed-effects meta-analysis*

Our meta-analysis of the relationship between SGR of fish and diet-P content was designed to answer three questions. First, does diet-P content limit growth of fish averaged across all studies? Such P limitation would be indicated by a statistically significant, positive weighted-mean linear coefficient in the quadratic model. Second, does high diet-P content inhibit growth of fish averaged across all studies? Growth inhibition by high diet-P would be indicated by a statistically significant, negative weighted-mean quadratic coefficient in the quadratic model. Last, we asked whether habitat (freshwater or marine) or trophic guild (herbivore, predator, or omnivore) influenced any of the coefficients in the quadratic model.

To address these questions, we conducted a mixed-effects meta-analysis of the quadratic equations (SGR vs. diet %P) following Gurevitch and Hedges (2001), with modification of the regression coefficients following Becker and Wu (2007). Diet-P level was a fixed effect and study was a random effect. We calculated weighted-mean coefficients and 95% confidence intervals for all studies combined, for habitat groups, and for trophic groups. We used nonparametric bootstrapping to estimate the bias-corrected 95% confidence intervals for each analysis using the "bca" option in "boot.ci" in the R package "boot" (Davison and Hinckley 1997, Canty and Ripley 2012). Briefly, we resampled the populations of weighted coefficients and mixed-effects variance 5000 times to estimate weighted-mean coefficients for each regression coefficient and grouping factor.

### *Model selection*

We also used an information-theoretic approach (Burnham and Anderson 2002) to identify likely candidate models for four parameters: peak SGR, optimum diet %P, the linear coefficient in the quadratic model, and the quadratic coefficient in the quadratic model. Instead of using raw values, we used the weighted effects from the meta-analysis. Model selection was conducted using the "dredge" function in the R package "MuMIn" (Bartoń 2013). Models were ranked by $\mathrm{AIC_c}$ scores and those with $\Delta\mathrm{AIC_c} < 2$ were considered the most likely models.

The model selection approach begins with identifying a hypothetical global model. We erred toward inclusivity by including as many interaction terms as possible. Empirical evidence and theory suggested that interactions existed among many of our predictor variables. For example, fish growth rates are known to vary with body size and temperature (Brett 1979). All three factors are thought to influence the P content of heterotrophs (Gillooly et al. 2002, Woods et al. 2003). Furthermore, preliminary data exploration indicated that relationships between several key parameters differed between freshwater and marine fishes.

## Results

### *The data set*

Our initial literature search identified 175 experiments in 148 publications. Of these, data from 124 experiments did not meet our criteria for inclusion. Many of these excluded studies either had fewer than four diet-P levels or confounded diet treatments, but 29 showed monotonic responses to diet-P level, and 32 studies had poor fits to the quadratic relationship ($R^2 < 0.5$), negative linear coefficients, or positive quadratic coefficients (see Appendix B). Of the studies meeting all of our initial criteria, one was excluded because the diet-P content at

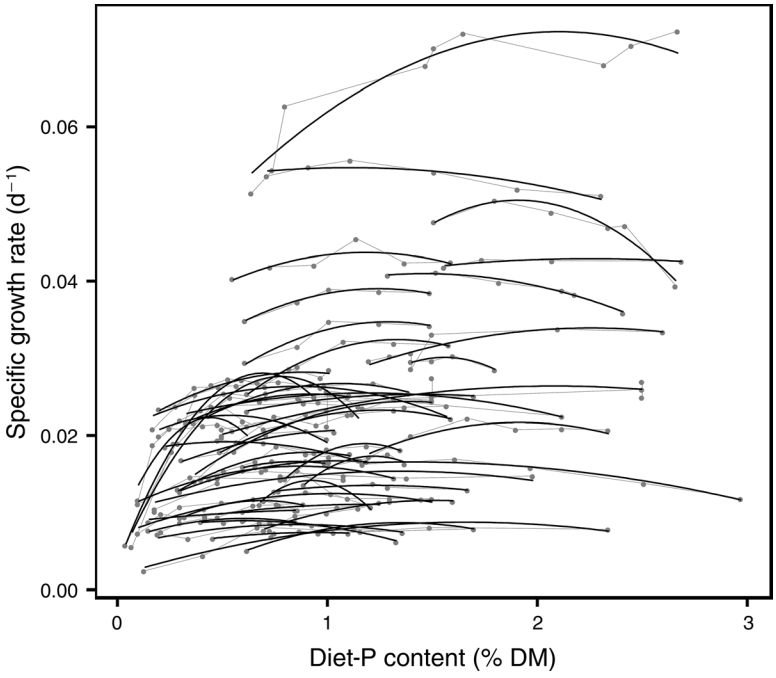


FIG. 1. Responses of specific growth rate (SGR) to diet %P treatments (with P expressed as percentage of dry mass of the diet, DM) across all experimental studies included in the SGR meta-analysis ($n = 49$ studies, 28 fish species). Fitted curves are the quadratic relationship.

which peak growth occurred (4.5%) was anomalously high ("high ash" treatments in Chaimongkol and Boonyaratpalin 2001), and one was excluded as an outlier based on a normal Q–Q plot of the quadratic coefficients (Huynh and Nugegoda 2011). The final data set included SGRs across diet-%P treatments for 49 experiments on 28 species in 16 families (Appendix C). Of these, body-%P was reported across diet-P treatments in 27 separate experiments on 20 species in 12 families.

The distribution of experiments was uneven across families, habitats, and trophic groups (Appendix C). Of the experiments analyzed, 59% used species in the families Salmonidae, Cyprinidae, Cichlidae, or Ictaluridae. A majority of the 49 experiments (61%) involved freshwater taxa. Two-thirds of the experiments were conducted with species classified as predators. In the 49 experiments, 27% of the species were classified as omnivores and 6% were classified as herbivores (three studies). The life stage of fish used in the experiments was predominantly newly hatched or juvenile. Initial wet body mass of fish ranged from 0.3 to 300 g. Experimental water temperatures also varied widely (8.8–30.0°C). The median duration of experiments was 62 d (range 28–182 d).

### *Phylogeny and phylogenetic signal*

Blomberg's $K$ computed using the BEAST time-calibrated tree (Appendix A) was not significant for peak SGR ($K = 0.78$, $P = 0.13$), optimum diet %P ($K = 0.92$, $P = 0.20$), the linear coefficient of the quadratic relationship ($K = 0.90$, $P = 0.87$), the quadratic coefficient of the quadratic relationship ($K = 0.87$, $P = 0.84$), body-P content at peak SGR ($K = 0.98$, $P = 0.33$), or $1/H$ ($K = 1.02$, $P = 0.67$). Overall, these results indicated that the data set lacked a strong phylogenetic signal (i.e., no statistically significant $K$ values).

### *Mixed-effects meta-analysis*

The mixed-effects meta-analysis supported a quadratic fit to the SGR vs. diet-P relationship across studies (mean with 95% CI: for the intercept, 0.004, 0.009–0.013; for the linear coefficient, 0.020, 0.025–0.033; for the quadratic coefficient, −0.018, −0.013 to −0.009). This finding is not particularly surprising, given our selection criteria (i.e., we excluded studies with monotonic relationships and poor quadratic fits). Yet, the results of this model are informative, indicating that growth was limited by diet-P concentration (i.e., the 95% CI of the weighted-mean linear coefficient did not overlap with zero). Moreover, the weighted-mean quadratic coefficient was significantly less than zero, indicating that high diet-P inhibited growth.

### *Peak SGR*

Peak SGR varied between 0.007 and 0.072 d$^{-1}$ across studies (Figs. 1 and 2a). Peak SGR did not differ between habitats ($t$ test, $P = 0.12$) or trophic groups (Type II ANOVA, $P = 0.21$). Weighted-mean peak SGR

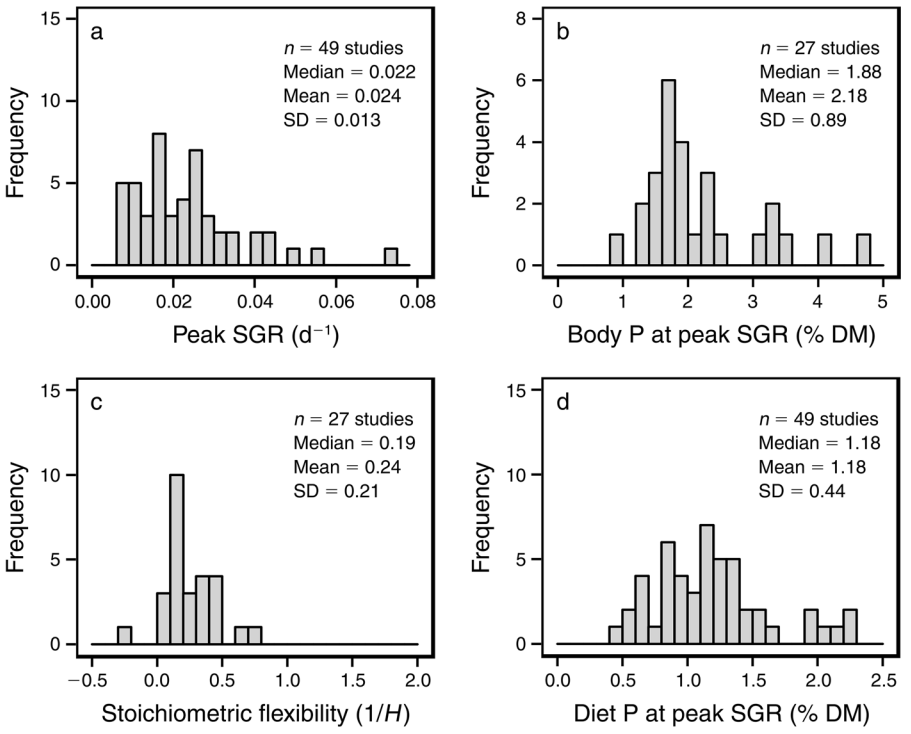


FIG. 2. Histograms showing frequency distributions (number of studies) of (a) peak specific growth rate (SGR), (b) body %P (percentage of body dry mass) at peak SGR, (c) stoichiometric flexibility ($1/H$, the inverse of homeostatic control of body %P), and (d) diet %P (percentage of diet dry mass) at peak SGR.

also did not differ among habitats or trophic groups. Peak SGR was negatively related to geometric mean body mass in grams (hereafter, mass; $\ln \text{SGR, d}^{-1} = -0.15(\ln(\text{wet mass})) - 3.48$; $R^2 = 0.19$, $P < 0.001$) and was positively related to temperature ($\ln \text{SGR, d}^{-1} = 0.05(^\circ\text{C}) - 4.96$; $R^2 = 0.23$, $P < 0.001$).

The two most likely ($\Delta\text{AIC}_c < 2$) models of peak SGR contained habitat, mass, optimum diet %P, and temperature ($R^2 = 0.58$–$0.59$; Table 1). As expected, peak SGR increased with temperature and decreased with mass. Peak SGR increased with optimum diet %P only in marine taxa. There was one likely model of peak SGR for the data subset with body-P ($R^2 = 0.73$; Table 2). Peak SGR decreased with mass and increased with temperature as in the full data set. An interaction between stoichiometric flexibility and body-P indicated

TABLE 1. Global models (GM) and candidate models with $\Delta_i \text{AIC}_c < 2$ for peak growth (PG) of the specific growth rate (SGR), optimum diet %P (OP), and linear and quadratic coefficients using the full data set in a meta-analysis of aquaculture experiments testing effects of diet %P on fish growth rate (49 studies, 28 species).

| Parameter | Rank | Model | $R^2$ | $\text{AIC}_c$ | $\Delta_i \text{AIC}_c$ | $w_i$ |
|---|---|---|---|---|---|---|
| Peak SGR | GM | Hab × M × T × OP | | | | |
| | 1 | Hab − M − OP + T + Hab × OP | 0.584 | 50.591 | 0.000 | 0.267 |
| | 2 | Hab − M − OP + T + Hab × OP + M × T | 0.593 | 52.401 | 1.810 | 0.108 |
| Optimum diet %P | GM | Hab × M × PG × T | | | | |
| | 1 | Hab + PG + Hab × PG | 0.253 | 40.570 | 0.000 | 0.257 |
| | 2 | Hab − PG + T + Hab × PG | 0.263 | 42.540 | 1.969 | 0.096 |
| Linear coefficient | GM | M + Hab × OP × PG × T | | | | |
| | 1 | M + PG | 0.203 | 119.043 | 0.000 | 0.115 |
| | 2 | PG | 0.155 | 119.563 | 0.520 | 0.089 |
| | 3 | M − OP + PG | 0.224 | 120.213 | 1.169 | 0.064 |
| | 4 | −OP + PG | 0.177 | 120.648 | 1.604 | 0.052 |
| | 5 | M + PG + T + PG × T | 0.255 | 120.825 | 1.782 | 0.047 |
| Quadratic coefficient | GM | M +Hab × OP × PG × T | | | | |
| | 1 | M − OP + PG | 0.353 | 119.984 | 0 | 0.142 |
| | 2 | −OP + PG | 0.313 | 120.465 | 0.481 | 0.111 |
| | 3 | M + OP + PG − OP × PG | 0.365 | 121.687 | 1.703 | 0.061 |

*Notes:* Model abbreviations are Hab, marine or freshwater habitat; M, body mass; and T, temperature. All predictors except temperature were ln-transformed. The quadratic coefficient was multiplied by −1 prior to transformation.

TABLE 2. Global models (GM) and candidate models with $\Delta_i$ $AIC_c < 2$ for peak SGR (PG), optimum diet %P (OP), and linear and quadratic coefficients using the subset of studies with body-P data (27 studies, 20 species).

| Parameter | Rank | Model | $R^2$ | $AIC_c$ | $\Delta_i$ $AIC_c$ | $w_i$ |
|---|---|---|---|---|---|---|
| Peak SGR | GM | $M + T + Hab \times BP \times 1/H \times OP$ | | | | |
| | 1 | $-BP - M - 1/H + T + BP \times 1/H$ | 0.725 | 28.989 | 0.000 | 0.338 |
| Optimum diet %P | GM | $M + Hab \times PG \times T + Hab \times BP \times 1/H \times PG$ | | | | |
| | 1 | $BP + T$ | 0.355 | 16.316 | 0.000 | 0.088 |
| | 2 | $BP + PG$ | 0.335 | 17.096 | 0.780 | 0.059 |
| | 3 | $-BP - PG + T + BP \times PG$ | 0.474 | 17.503 | 1.186 | 0.049 |
| | 4 | $BP + M + PG$ | 0.396 | 17.680 | 1.363 | 0.044 |
| | 5 | T | 0.233 | 17.997 | 1.681 | 0.038 |
| | 6 | $BP + M + T$ | 0.384 | 18.196 | 1.880 | 0.034 |
| | 7 | $BP + T + PG$ | 0.384 | 18.199 | 1.882 | 0.034 |
| Linear coefficient | GM | $M + Hab \times OP \times PG \times T + Hab \times BP \times 1/H$ | | | | |
| | 1 | PG | 0.226 | 65.722 | 0.000 | 0.115 |
| | 2 | $1/H + PG$ | 0.670 | 67.010 | 1.288 | 0.060 |
| Quadratic coefficient | GM | $M + Hab \times OP \times PG \times T + Hab \times BP \times 1/H$ | | | | |
| | 1 | $-OP + PG$ | 0.221 | 68.434 | 0.000 | 0.073 |
| | 2 | [intercept term only] | 0.000 | 69.559 | 0.125 | 0.041 |
| | 3 | $1/H$ | 0.081 | 69.942 | 1.509 | 0.034 |
| | 4 | $1/H - OP + PG$ | 0.266 | 69.988 | 1.555 | 0.033 |
| | 5 | PG | 0.078 | 70.010 | 1.577 | 0.033 |

*Notes:* Model abbreviations are Hab, marine or freshwater habitat; M, mass; T, temperature; BP, body-P; and $1/H$, stoichiometric flexibility. All predictors except temperature were ln-transformed. The quadratic coefficient was multiplied by $-1$ prior to transformation (as in the Table 1 footnote).

that peak SGR increased with stoichiometric flexibility, particularly for high-P fishes. This model did not include habitat.

### *Body-P content at peak SGR and stoichiometric homeostasis*

Body-P content at peak SGR varied widely among studies (0.9–4.6% P; Fig. 2b) but did not vary between habitats ($t$ test, $P = 0.77$) or among trophic groups (Type II ANOVA, $P = 0.96$). Body-P content at peak SGR was not linearly related to mass ($P = 0.46$) or peak SGR ($P = 0.67$). The weighted-mean body-P content at peak SGR did not vary between habitats or among trophic groups.

Body-P content increased with diet-P content in 19 of 27 studies, although a few studies showed a saturating (i.e., nonlinear) relationship (Fig. 3). In one study (Uyan

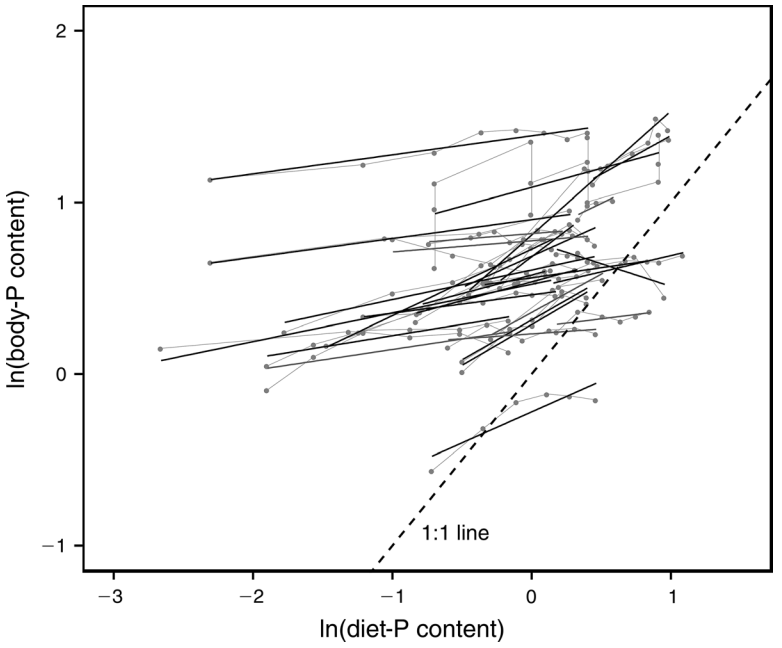


FIG. 3. Responses of ln-transformed body-P content (originally measured as percentage dry mass) to ln-transformed diet P treatments (originally measured as percentage dry mass) across the subset of experimental studies that reported body-P data ($n = 27$ studies). Black regression lines show relationships with slopes that are significantly higher than zero ($P < 0.1$); slopes of gray regression lines are not different from zero. The dashed line depicts a 1:1 relationship.

et al. 2006), body-P content declined with increasing diet-P content; however, diet-P content in this study was relatively high (1.2–2.6%; the third quartile of diet-P across all studies was 1.3%). Data from this study were not included in subsequent analyses of stoichiometric flexibility. In the remaining seven studies, the slope of the relationship between body-P and diet-P (hereafter, stoichiometric flexibility, $1/H$) was not significantly greater than zero ($P > 0.1$). Stoichiometric flexibility ($1/H$) varied from 0.06 to 0.72 (–0.26 in Uyan et al. [2006]; Fig. 2c), corresponding to a range in $H$ of 1.39 to 16.67.

### *Diet-P at peak SGR: optimum diet %P*

Optimum diet %P, the diet-P content at which SGR peaked, ranged from 0.4% to 2.3% and did not differ between habitats ($t$ test, $P = 0.11$) or among trophic groups (Type II ANOVA, $P = 0.80$; Fig. 2d). Optimum diet %P estimates were well constrained for most studies (Appendix D). In 75% of the studies, the 95% confidence interval range for optimum diet %P (i.e., the difference between the upper and lower 95% confidence intervals) was less than half of the range in diet-P treatments.

The weighted-mean optimum diet %P was also not significantly different among trophic groups or habitats. Optimum diet %P was not linearly related to mass ($P = 0.24$) or temperature ($P = 0.16$), but increased with peak SGR (ln optimum diet %P $= 0.26$(SGR, $d^{-1}$) $+ 1.10$; $R^2 = 0.13$, $P = 0.01$). Optimum diet %P did not vary with stoichiometric flexibility ($P = 0.26$), but increased with body-P content (ln optimum diet %P $= 0.39$(Fish %P) $- 0.06$; $R^2 = 0.15$, $P = 0.04$).

The two most likely models of optimum diet %P using the full data set included an interaction between peak SGR and habitat ($R^2 = 0.25$ and 0.26; Table 1). Optimum diet %P increased with peak SGR for marine taxa only. In the data subset with body-P values, there were seven highly likely models of optimum diet %P ($R^2 = 0.23$–$0.47$). Optimum diet %P increased with peak SGR and body-P content and was particularly high in rapidly growing, P-rich fishes. Optimum diet %P also increased with temperature and mass (although not in the full data set). The most likely models for this data set did not include habitat.

### *Influence of diet-P on SGR: severity of P limitation*

There were five highly likely models of the linear coefficient in the quadratic model ($R^2 = 0.16$–$0.26$; Table 1). The severity of P limitation of growth increased with peak SGR. The two likely models for the data subset with fish-P values ($R^2 = 0.23$ and 0.67; Table 2) indicate that the severity of P limitation increased with peak SGR and with stoichiometric flexibility. Adding stoichiometric flexibility to the model containing only peak growth greatly increased the proportion of variation explained (Table 2).

### *Influence of diet-P on SGR: severity of P inhibition*

The three likely models of the quadratic coefficient from the quadratic model ($R^2 = 0.31$–$0.37$; Table 1) indicate that the severity of P inhibition increased with both peak SGR and mass, but it decreased with optimal diet %P. For the data subset with fish-P values, there were five likely models of the quadratic coefficient ($R^2 = 0$–$0.27$; Table 2). Surprisingly, the second most likely model contained only the intercept. For this data subset, the severity of P inhibition increased with peak SGR and decreased with optimum diet %P. The severity of P inhibition also increased with stoichiometric flexibility.

## Discussion

Our meta-analysis revealed several patterns in the relationships among P imbalance, body-P content, and growth in fishes. We found evidence for both limitation and inhibition of growth by P, as well as a role for flexible body-P content in ameliorating these negative effects. Relatively shallow relationships between growth rate and diet-P level (i.e., a "dull" stoichiometric "knife-edge"), coupled with surprisingly flexible body-P content in fishes, is consistent with fish using flexible body-P pools (presumably dominated by bone P) to buffer imbalances between P intake and demand.

Analysis of the collated data set provided ample evidence for dietary P limitation under experimental conditions of relatively high food intake. Clearly, this phenomenon is well documented in aquaculture applications, but our results can also shed light on the potential for P limitation of fish growth under natural feeding conditions. In general, wild fish are believed to grow at well below peak rates due to strong energy limitation (Schindler and Eby 1997). However, recent studies have suggested that wild fish may be susceptible to P limitation when making large investments in P-rich tissues or when feeding at high rates (or both). For example, periods of rapid potential growth (e.g., in young-of-year) or consumption of high-volume, nutrient-poor diets such as detritus, algae, or plants, or low-P invertebrates, may be associated with P-limitation of fish growth under natural conditions (Hood et al. 2005, Malzahn et al. 2007, Boersma et al. 2008). Our results integrate the robust experimental support for such predictions. Rapidly growing and P-rich fishes had higher diet-P requirements at peak growth. The interaction between peak SGR and body-P indicated that both SGR and body-P content (i.e., the P content of new tissue) played a role in shaping P demand. Fishes therefore require P both to sustain rapid growth, presumably for RNA, and to create P-rich tissues (e.g., bone) that are not directly associated with growth rate.

Surprisingly, the optimum diet %P was well above the P content of many constituents of natural fish diets (e.g., algae, detritus, and macroinvertebrates). The mean optimum diet P was 1.2% and the interquartile range was 0.9% to 1.4%. Assuming a diet-C content of 45%, this equates to a mean optimal diet C:P ratio of 97

(interquartile range 83 to 129; molar), which is much lower than the mean C:P of algae and aquatic detritus and lower even than the C:P of most macroinvertebrates (Elser et al. 2000, Sterner and Elser 2002). The optimal diet C:P calculated here is equivalent to a threshold elemental ratio ($TER_{C:P}$), either side of which fish growth is limited by either C or P in their diet (Frost et al. 2006). Note that TERs do not incorporate negative effects of high P intake. Any $TER_{C:P}$ increases as consumption declines because a greater proportion of C is used for basal metabolic costs instead of being coupled with P for growth (Sterner and Robinson 1994). The mean optimal diet C:P that we found strongly suggests that fish growth becomes increasingly P limited as consumption rate of many natural diets increases (i.e., when food is abundant) and that this is particularly acute with diets low in P. Moreover, diet-P may be important in regulating growth and condition even when growth is not strictly limited by P. Phosphorus plays a key role in the absorption and utilization of energy and other nutrients contained in food (e.g., Xie et al. 2011). This role suggests that suboptimal diet-P levels may influence growth by mediating energy acquisition even when energy is sufficient.

Surprisingly, the severity of P limitation was not related to body-P content, as predicted by stoichiometric theory, but was related to SGR. Our findings support similar work with *Daphnia* (the group for which the most information is available; Seidendorf et al. 2010, Hood and Sterner 2014). Yet, this comparison highlights differences between invertebrates and vertebrates. For small invertebrates, the RNA-P pool is the dominant driver of P demand (Kyle et al. 2006). In contrast, the RNA-P pool is a small proportion of the total P pool in vertebrates such as fish (Gillooly et al. 2005). Instead, their largest single pool is associated with bone. This structural material plays the dominant role in shaping the P demand of fishes, their response to P-deficient diets, and their stoichiometric flexibility.

Another significant result was the abundant evidence for an inhibitory effect of high diet-P content on fish growth, with taxa exhibiting a hyperbolic (and not saturating) growth response to diet-P level. Identifying potential physiological mechanisms for P inhibition of fish growth was beyond the scope of our analysis (for a discussion, see Boersma and Elser 2006), but we were able to test some related predictions. Boersma and Elser (2006) hypothesized that such responses should be particularly acute in taxa adapted to low-P food or with low body-P content or low growth rates. In our analysis, the severity of growth inhibition indeed declined as optimum diet %P increased, indicating that taxa requiring relatively high-P diets were less likely to suffer negative consequences of high P intake. In contrast, the severity of growth inhibition was not related to body P content and actually increased with SGR, suggesting that dilution of excess P in new growth is not an important mechanism for managing excess P in fish. Such mechanisms may be less important in vertebrates, for which bone represents a dominant P sink. The smaller body-P data set prevented firm conclusions, but there was wide variation in body-P flexibility among taxa, with most studies showing a log-linear relationship between body-P content and diet-P, while a few exhibited plateau or hyperbolic relationships. It seems likely, therefore, that fish use a combination of excretion and buffering of the bone-P pool (the relative metabolic costs of which are unknown) to mitigate the consequences of excess P. How vertebrates control these processes to balance limitation vs. inhibition by P deserves further examination.

The degree of stoichiometric flexibility varied widely among taxa. Although there were some cases of strict homeostasis (i.e., no change in body-P content with diet-P content), many fishes were stoichiometrically more flexible than invertebrates and some were as flexible as algae (Persson et al. 2010). Variation in body-P with diet-P can be attributed primarily to loss of P bound in bone (e.g., Watanabe et al. 1980). Suboptimal diet %P can lead to a decline in bone %P and eventual bone deformities (e.g., Roy and Lall 2003). Fishes with high peak SGR were the most stoichiometrically flexible, suggesting that fishes with the highest P demand used the bone-P pool to allow growth on low-P diets. Furthermore, the degree of stoichiometric flexibility was a predictor of both P limitation and P inhibition. Taken together, this suggests that P in bone is used to buffer P imbalance, albeit at a cost. In fact, the optimum dietary %P for peak growth is often lower than that for body-P content (Xie et al. 2011). Thus, P in bone represents a P reservoir, a determinant of body-P requirements, and an important component of fish function. It is possible that the dual role of P in bone in fish P budgets (i.e., both a determinant of P requirements and a buffer against P imbalance) explains the lack of a relationship between the severity of P limitation and body-P content.

Our results also revealed some subtle and intriguing differences between freshwater and marine taxa in their coupling of dietary P and growth rate. Peak SGR of marine species was positively related to optimum diet %P, unlike freshwater taxa, which showed a wide range in peak SGRs and no strong relationship with optimum diet %P. Unfortunately, this difference could not be explained by variation in body P content between the two groups, leaving a mechanism for such a difference unexplained. However, differences in physiology and environment between marine and freshwater fishes suggest that differences in their P allocation and conservation might exist and that further research on this topic may be warranted.

The importance of accounting for potential phylogenetic signal in meta-analytical response variables has been demonstrated recently by a detailed comparison between results of traditional and phylogenetically

corrected meta-analysis of the same data sets (Chamberlain et al. 2012). We found no evidence for phylogenetic signal in the treatment effects in our analysis. Although this result indicated that our data could be considered statistically independent (with respect to phylogeny) and that a traditional meta-analysis was appropriate, it was somewhat surprising (see Hendrixson et al. 2007). One potential explanation is that growth and stoichiometric traits in the diverse species that we included are under strongly differential selection, leading to adaptations that are not correlated with the phylogeny (i.e., homoplasy). A more mundane reason is simple measurement error, either in the phylogenetic tree or the trait values themselves (Blomberg et al. 2003). Nevertheless, the potential for convergent evolution of stoichiometric traits in the lineages included in our analysis seems strong (e.g., via multiple freshwater invasions).

Some caveats also have to be considered in our analysis. First, the species in our meta-analysis, while phylogenetically diverse, are not a random subset of fish taxa. Presumably, many were originally selected for characteristics such as rapid growth, as well as other traits deemed desirable for aquaculture (e.g., tolerance of high densities, disease resistance), which may have biased our results directly or indirectly. For example, bias toward high growth rates would be expected to drive up the potential for P limitation. Second, many populations in our analysis presumably also have been under artificial selection for the same traits, which may have biased our results further. However, these implicit limitations of the data set are countered by one advantage. Publication bias was probably not a significant problem in our data set, given that a priori thresholds for a significant result were unlikely to be an important criterion for their original publication.

In summary, several conclusions can be drawn from our meta-analysis. First, fish show a high degree of flexibility in body-P content. Flexibility in body-P may be a general feature of vertebrate stoichiometry that requires incorporation into theory, particularly because more flexible species appear to achieve higher growth rates. Second, despite flexibility in body-P, we found significant sensitivity of fish growth to both low and high diet-P levels. Diet-P levels below 1.2% were limiting at the high ration levels used in the experiments, suggesting that ecologically relevant P content in natural food resources can limit fish growth at high ingestion rates. The ecological significance of growth inhibition by high diet-P is less clear, but our results indicate that stoichiometric flexibility does not completely circumvent any costs of excess P intake. Although no organism is immune from such P imbalances, our results suggest that fishes, and perhaps other vertebrates, use body-P pools to buffer mismatches, giving rise to a relatively "dull" stoichiometric "knife-edge" in the relationships between their growth rate and dietary P level.

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## Supplemental Material

### Appendix A

A description of the methods used to construct the phylogeny of fish species included in the meta-analysis and a plot of the resulting time-calibrated phylogeny (*Ecological Archives* E095-240-A1).

### Appendix B

Responses of specific growth rate (SGR) to diet %P treatments across experimental studies excluded from the SGR analysis (*Ecological Archives* E095-240-A2).

### Appendix C

Summary of publications included in the meta-analysis of dietary P-growth coupling in fish (*Ecological Archives* E095-240-A3).

### Appendix D

Confidence intervals (95%) for optimal dietary %P for each study included in the meta-analysis (*Ecological Archives* E095-240-A4).