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Nutrient limitation, bioenergetics and stoichiometry: A new model to predict elemental fluxes mediated by fishes

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Abstract

1. Energy flow and nutrient cycling dictate the functional role of organisms in ecosystems. Fishes are key vectors of carbon (C), nitrogen (N) and phosphorus (P) in aquatic systems, and the quantification of elemental fluxes is often achieved by coupling bioenergetics and stoichiometry. While nutrient limitation has been accounted for in several stoichiometric models, there is no current implementation that permits its incorporation into a bioenergetics approach to predict ingestion rates. This may lead to biased estimates of elemental fluxes.
2. Here, we introduce a theoretical framework that combines stoichiometry and bioenergetics with explicit consideration of elemental limitations. We examine varying elemental limitations across different trophic groups and life stages through a case study of three trophically distinct reef fishes. Further, we empirically validate our model using an independent database of measured excretion rates.
3. Our model adequately predicts elemental fluxes in the examined species and reveals species- and size-specific limitations of C, N and P. In line with theoretical predictions, we demonstrate that the herbivore *Zebrasoma scopas* is limited by N and P, and all three fish species are limited by P in early life stages. Further, we show that failing to account for nutrient limitation can result in a greater than two-fold underestimation of ingestion rates, which leads to severely biased excretion rates.
4. Our model improved predictions of ingestion, excretion and egestion rates across all life stages, especially for fishes with diets low in N and/or P. Due to its broad applicability, its reliance on many parameters that are well-defined and widely accessible, and its straightforward implementation via the accompanying R-package

FISHFLUX, our model provides a user-friendly path towards a better understanding of ecosystem-wide nutrient cycling in the aquatic biome.

KEYWORDS
bioenergetics, fish, ingestion, nitrogen, nutrient cycling, nutrient limitation, phosphorus, stoichiometry

1 | INTRODUCTION

Internal biological processes of consumer species, such as growth, respiration and excretion are important drivers of ecosystem-scale biogeochemical cycles (Welti et al., 2017). To survive, individuals need to gather resources from the environment and, in doing so, transfer energy and nutrients within and across ecosystems (Brown, Gillooly, Allen, Savage, & West, 2004; Mackenzie, Ver, Sabine, Lane, & Lerman, 1993). Therefore, the quantification of energy and nutrient fluxes in ecosystems is affected by our ability to understand how energy and materials are utilized and transformed at the individual level (Allen & Gillooly, 2009; Allgeier, Yeager, & Layman, 2013; Kitchell et al., 1974; Sterner & Elser, 2002).

In many aquatic ecosystems, fishes account for most of the heterotrophic biomass (Odum & Odum, 1955; Vanni, 2002) and contribute substantially to the storage and flux of carbon (C), nitrogen (N) and phosphorus (P) (Allgeier, Layman, Mumby, & Rosemond, 2014; Barneche et al., 2014; Burkepile et al., 2013; McIntyre et al., 2008; Vanni, 2002). Storage is primarily dictated by food that is assimilated and allocated to growth, which ultimately underpins critical ecosystem services (e.g. finfish fisheries). Fluxes are derived from assimilated (respired carbon and excreted nutrients) and non-assimilated food (egested organic waste; Schreck & Moyle, 1990), and they can have important effects on ecosystem processes, such as primary production (Allgeier et al., 2013; Capps & Flecker, 2013; McIntyre et al., 2008). Disentangling how fishes partition ingested elements into biomass and waste products is therefore key to linking individual-level physiology to ecosystem-level processes, which are of inherent human interest (Allen & Gillooly, 2009; Anderson, Hessen, Elser, & Urabe, 2005; Barneche & Allen, 2018; Hessen, Ågren, Anderson, Elser, & De Ruiter, 2004; Hou et al., 2008).

Ecological stoichiometry provides a theoretical framework to understand how consumers partition C, N and P (Sterner & Elser, 2002). On the basis of the conservation of mass, the material ingested by consumers equals the sum of biomass accumulation and waste products such as respired carbon, excreted nutrients and egested organic material. Furthermore, stoichiometric theory predicts that the ratio of recycled elements depends on the elemental composition of the consumer body, diet and the gross growth efficiency of the limiting element (Frost et al., 2006; Sterner, 1990). Thus, given known ingestion rates, stoichiometric mass balance models allow for the prediction of fish excretion rates (Barneche & Allen, 2015; Kraft, 1992; Schindler & Eby, 1997). Ingestion rates can be approximated using empirical relationships with body mass and temperature (e.g. Elliott & Persson, 1978; El-Sabaawi, Warbanski, Rudman, Hovel, & Matthews, 2016), but these estimates are highly species-specific, require extensive lab experiments, and may not reflect fish ingestion rates in the wild.

Alternatively, ingestion rates can be estimated using bioenergetic models. In fact, there is a rich history of bioenergetic modelling approaches to estimate energy allocation in fishes under the assumption that they are limited by energy (C; e.g. the ‘Wisconsin model’, Hanson, Johnson, Schindler, & Kitchell, 1997; Kitchell et al., 1974 and the ‘Dynamic Energy Budget model’, Kooijman, 2010). Combined with elemental stoichiometry, bioenergetic models therefore provide a conceptual basis to predict how fishes partition energy and nutrients into growth, metabolism and waste (Deslauriers, Chipps, Breck, Rice, & Madenjian, 2017; Kraft, 1992; Schindler & Eby, 1997; Schreck & Moyle, 1990). This approach has been widely used to estimate ingestion rates, given known growth rates in wild fish populations (especially via the Fish Bioenergetics software; Deslauriers et al., 2017). Nutrient cycling predictions are then made by combining modelled ingestion rates based on energetic needs, assimilation efficiencies and nutrient stoichiometry of both a fish's body and diet (Allen & Gillooly, 2009; Anderson et al., 2005; Kraft, 1992; Schindler & Eby, 1997).

Although useful and successfully implemented (Deslauriers et al., 2017), this approach is limited in its application to fishes that are limited by C. This can be the case, especially for trophic groups that feed on nutrient-rich prey (e.g. Schindler & Eby, 1997); yet, many fish species in low trophic levels may be limited by N or P because their diets contain lower nutrient levels than their body tissues (McIntyre et al., 2008; Schindler & Eby, 1997). Thus, applying the traditional approach of combining stoichiometry and bioenergetics (Kraft, 1992) to fish species that are limited by N or P normally results in biologically implausible predictions of excretion rates. Indeed, there is mounting evidence that fishes can be limited by nutrients, rather than energy (Benstead et al., 2014; El-Sabaawi et al., 2016; Hood, Vanni, & Flecker, 2005; Moody, Lujan, Roach, & Winemiller, 2019). While, negative predicted excretion rates can provide evidence for nutrient limitation (e.g. Hood et al., 2005), they do not aid our understanding and prediction of realistic elemental fluxes in communities where nutrient-limited species are prevalent. Thus, although many stoichiometric models take into account nutrient limitation (e.g. El-Sabaawi et al., 2016; Guariento, Luttbeg, Carneiro, & Caliman, 2018; Moody et al., 2018, 2019 Sterner, 1990), there is presently no solution for integrating nutrient limitation into bioenergetic models that quantify ingestion rates. As fishes in low trophic levels often account for a significant proportion of biomass (e.g. Graham et al., 2017) and represent important vectors of nutrients, a new approach is needed to accurately predict elemental fluxes in the absence of known ingestion rates.

Here, we present a theoretical framework (and a companion R package for its implementation: FISHFLUX) to predict elemental fluxes in fishes that combines bioenergetics and ecological stoichiometry while directly accounting for N and P limitation, alongside C limitation. The proposed model framework predicts ingestion rates based on the needs of a fish at a certain size for all three elements and a known growth rate. We test our framework via a case study of three trophically distinct coral reef fish species: the herbivore *Zebrasoma scopas* (family Acanthuridae), the omnivore *Balistapus undulatus* (family Balistidae) and the carnivore *Epinephelus merra* (family Serranidae). We also validate our model against independent empirical excretion estimates for our three fish species. Furthermore, we test whether fishes in different trophic levels and life stages are limited by different elements and hypothesize that fishes at low trophic levels are limited by N or P rather than C. Finally, we posit that, by building on existing approaches, our framework considerately improves the prediction of key processes such as ingestion and excretion in the case of strong nutrient limitation, as compared to models that only consider C limitation.

2 | MATERIALS AND METHODS

2.1 | Theoretical framework

Carbon, nitrogen and phosphorus (CNP, expressed in grams) are the three chemical elements considered in our model. The approach applies a mass-balance framework based on ecological stoichiometry and the metabolic theory of ecology (Allen & Gillooly, 2009; Barneche & Allen, 2015; Brown et al., 2004; Sterner & Elser, 2002). Further, the approach relies on the growth trajectory of natural fish populations. The proposed model has four main steps (Figure 1): (a) The minimal required ingestion or minimal supply rate of CNP is defined as the sum of CNP needed for a given growth increment and minimal inorganic flux (i.e. the minimal requirements of CNP needed for metabolism and the maintenance of the body stoichiometry). In this step, we also consider assimilation efficiency, which is defined as the capacity of an organism to assimilate C, N or P (input parameters of the model). (b) Ingestion is estimated based on the limiting element that is defined by the imbalance between the CNP composition of the minimal supply rate and that of the diet. (c) The egestion rate is then quantified according to the ingestion rate and the assimilation efficiencies of each element. (d) The residual CNP are allocated towards the total inorganic flux of CNP (i.e. the waste inorganic CNP that is produced from physiological transformation). For the sake of comparison with existing literature, we note that the inorganic flux of C is generally called total metabolic rate, whereas the inorganic fluxes of N and P are called excretion rates. Materials that are not assimilated are egested as organic waste. An overview of all main variables predicted by the model and input parameters that need to be specified by the user is given in Table 1, while other parameters mentioned in the text are fixed in the model. In the following sections, we detail each component of the model.

2.1.1 | Minimal supply rate

The first step of the model is an estimate of the minimal supply rate of elements (C, N and P) required per day for a given growth increment in an individual of a given size. The required CNP is the sum of the elements needed for body mass growth and overhead metabolic and maintenance costs (i.e. minimal inorganic flux). The minimal supply rate S_k (g/day) of the element $k = \{C, N, P\}$ can therefore be estimated as

$$S_k = \frac{\left(G_k + F_{0k}\right)}{a_k}, \tag{1}$$

where G_k, F_{0k} and a_k are element-specific growth rate (g/day), minimal inorganic flux (g/day) and assimilation efficiency (%), respectively.

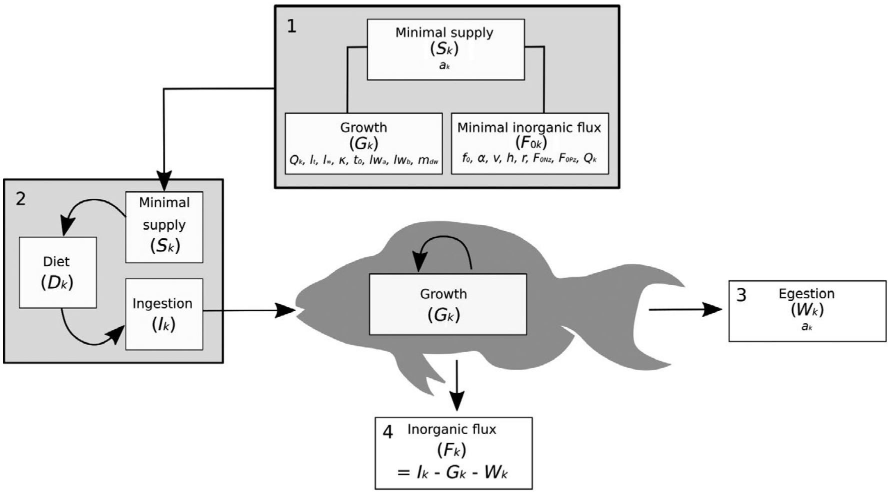

FIGURE 1 Conceptual diagram, explaining different model components. Required ingestion of C, N and P is calculated through the sum of elements needed for growth and minimal inorganic flux, taking into account the element-specific assimilation efficiencies, a_k (1). Based on the limiting element (due to the imbalance of food and the required CNP), the ingestion rate can be estimated (2). The ingested material is partitioned into egestion (3) and assimilation (body mass growth and flux (4)). The symbol of each component is indicated in between brackets. The input parameters needed to calculate the different variables are italicized. See Table 1 for a description of each parameter

TABLE 1 Overview of model parameters and variables, including input parameters, to be specified by the user of the model, which are indicated with ✳. Main output variables, predicted by the model are indicated with ▲. VBGC, von Bertalanffy growth curve

Symbol	Description	Unit
k	Index for element C, N or P	—
S_k ▲	Element-specific minimal supply rate	g/day
G_k ▲	Element-specific growth	g/day
F_{0k} ▲	Element-specific minimal inorganic flux	g/day
a_k ✳	Element-specific assimilation efficiency	—
l_t ✳	Total length of individual at time t	cm
t	Age	year
l_∞ ✳	Asymptotic adult length (VBGC)	cm
κ ✳	Growth rate parameter (VBGC)	year^{-1}
t_0 ✳	Age at settlement (VBGC)	year
lw_a ✳	Parameter length—weight relationship	g/cm
lw_b ✳	Parameter length-weight relationship	—
Q_k ✳	Element—specific body content percentage of dry mass	%
m_w	Wet body mass	g
F_{0Cr}	Resting metabolic rate	g/day
F_{0Cz}	Mass-specific turnover rate of C	$\text{g C g}^{-1}\text{ day}^{-1}$
F_{0Cs}	Rate of C spent in body mass growth	g/day
f_0 ✳	Metabolic normalization constant independent of body mass	$\text{g C g}^{-\alpha}\text{ day}^{-1}$
α ✳	Mass-scaling exponent	—
$m_{w\infty}$	Asymptotic wet mass of an adult individual	g
ϕ	Cost of growth	g C/g
θ ✳	Activity scope	—
v ✳	Environmental temperature	°C
h ✳	Trophic level	—
r ✳	Aspect ratio of caudal fin	—
F_{0Nz} ✳	Mass-specific turnover rate of N	$\text{g N g}^{-1}\text{ day}^{-1}$
F_{0Pz} ✳	Mass-specific turnover rate of P	$\text{g P g}^{-1}\text{ day}^{-1}$
m_{dw}	Ratio of dry mass and wet mass of fish	—
m_d	Dry body mass	g
D_k ✳	Element-specific diet content percentage of dry mass	%
I_k ▲	Element-specific ingestion rate	g/day
W_k ▲	Element-specific egestion rate	g/day
F_{rk} ▲	Element-specific residual inorganic flux	g/day
F_k ▲	Element-specific total inorganic flux	g/day

Growth

The aim of our model is to predict elemental fluxes of fishes in their natural environment. Therefore, we use growth rates that can be calculated from otolith analysis. In our model, we thus assume that there is enough food available to fulfil the observed growth pattern. We further use the von Bertalanffy growth curve (VBGC) to describe the growth trajectory (von Bertalanffy, 1957). Empirically, the VBGC is favourable because its parameters are statistically simple to obtain, easy to interpret, and are available for a large number of species (Morais & Bellwood, 2018). Body length, l_t (cm in total length, i.e. T.L.), at age t (year) is

$$l_t = l_\infty \left(1 - e^{-\kappa(t - t_0)}\right), \tag{2}$$

where t_0 is age at settlement, l_∞ is the asymptotic adult length (i.e. length when growth rate is 0), and κ is a growth rate parameter (year^{-1}; von Bertalanffy, 1957). With this equation, we can quantify the age of a fish of a certain size. Then, by adding one day to that age, we can also approximate the amount a fish will grow in one day. Using length–weight relationships and wet-to-dry mass conversion constants from the literature and FishBase (Froese & Pauly, 2018), we can finally calculate total growth rate (i.e. G) expressed in dry mass (g/day). Using element-specific body content percentages, Q_k, we calculate element-specific growth as:

$$G_k = \frac{Q_k}{100} G. \tag{3}$$

Minimal inorganic flux

Traditionally, the field metabolic rate, F_{0C}, has been studied more intensively than minimal excretion rates for N and P, F_{0N} and F_{0P}. As a consequence, we currently have a better understanding of how assimilated carbon is partitioned into body mass growth (G_C) and metabolic overhead costs (F_{0C}). For instance, we know that F_{0C} predictably scales with individual wet body mass, m_w (g; Hou et al., 2008):

$$\begin{aligned} F_{0C} &= \theta F_{0Cr} \\ &= \theta \left(F_{0Cz} m_w + F_{0Cs}\right) \\ &= \theta \left(f_0 m_{w\infty}^{\alpha - 1} m_w + \phi G\right), \end{aligned} \tag{4}$$

where F_{0Cr} is the resting metabolic rate (g C/day), F_{0Cz} is the mass-specific turnover rate ($\text{g C g}^{-1}\text{ day}^{-1}$), F_{0Cs} is the rate of carbon spent in body mass growth, and f_0 is a metabolic normalization constant that is independent of body mass ($\text{g C g}^{-\alpha}\text{ day}^{-1}$) and varies among fish taxa, environmental temperature and trophic level (Barneche et al., 2014; Barneche & Allen, 2018; Brown et al., 2004). α is a dimensionless mass-scaling exponent (generally between 0.5 and 1), $m_{w\infty}$ is the asymptotic mass of an individual, and ϕ is the energy expended to produce one unit of body mass (g C/g; hereafter the 'cost of growth'). In Equation 4, F_{0C} is defined as the sum of the resting metabolic rate, F_{0Cr}, and the active rate that sustains locomotion, feeding and other activities. We assume that $F_{0C} = \theta F_{0Cr}$ in the expression above, where θ is a dimensionless parameter referred to as 'activity scope', which is constrained to be >1 and less than the ratio between maximum metabolic rate and resting metabolic rate (Barneche & Allen, 2018; Hou et al., 2008).

The cost of growth, ϕ, varies substantially among fishes, and it may increase with environmental temperature, v, trophic level, h, and aspect ratio of caudal fin, r (a proxy for activity level; Froese & Pauly, 2018). Following Barneche and Allen (2018), the cost of growth can be calculated as

$$\ln\phi = \beta_0 + \beta_v v + \beta_h \ln h + \beta_r \ln(r+1), \tag{5}$$

where β_0 is a constant, β_v, β_h and β_r are respectively the model slopes for v, h and r. We note that h and r are two ecological variables that can be retrieved from FishBase (Froese & Pauly, 2018). For the purposes of our bioenergetic model, we use average, across-species estimates for β_0, β_v, β_h and β_r published in Barneche and Allen (2018).

Aside from inorganic fluxes of C, N and P will also be released at a minimal rate, even when they are limiting (Anderson et al., 2005; Sterner & Elser, 2002). The minimal inorganic flux of N and P can be experimentally measured as minimal excretion rates during starvation (Mayor et al., 2011). We can thus explicitly incorporate N and P turnover rates to estimate minimal inorganic flux of N and P (Anderson et al., 2005).

$$F_{0\mathrm{N}} = F_{0\mathrm{Nz}} \frac{Q_\mathrm{N}}{100} m_d, \quad \text{and} \tag{6}$$

$$F_{0\mathrm{P}} = F_{0\mathrm{Pz}} \frac{Q_\mathrm{P}}{100} m_d, \tag{7}$$

where $F_{0\mathrm{Nz}}$ and $F_{0\mathrm{Pz}}$ are nutrient-specific dry mass-specific turnover rates for N (g N g^{-1} day^{-1}) and P (g P g^{-1} day^{-1}), respectively, and m_d is the dry mass of the fish (g). Equations 6 and 7 assume that $F_{0\mathrm{Nz}}$ and $F_{0\mathrm{Pz}}$ remain constant during ontogeny.

2.1.2 | Ingestion

In our model, the quantification of ingestion rate is a two-step process. First, we define the minimal required ingestion of CNP by summing element-specific minimal supply rates S_k. Second, we approximate the actual ingestion rates by using ecological stoichiometric theory (Sterner & Elser, 2002). With known elemental stoichiometry of the diet (D_C, D_N, D_P) we can determine the limiting element as follows:

$$\text{limiting element} = \left\{ \begin{array}{lll} \mathrm{C}, & \text{if} & \frac{S_\mathrm{C}}{S_\mathrm{N}} > \frac{D_\mathrm{C}}{D_\mathrm{N}} \ \text{and} \ \frac{S_\mathrm{C}}{S_\mathrm{P}} > \frac{D_\mathrm{C}}{D_\mathrm{P}} \\ \mathrm{N}, & \text{if} & \frac{S_\mathrm{N}}{S_\mathrm{P}} > \frac{D_\mathrm{N}}{D_\mathrm{P}} \ \text{and} \ \frac{S_\mathrm{C}}{S_\mathrm{N}} < \frac{D_\mathrm{C}}{D_\mathrm{N}} \\ \mathrm{P}, & \text{otherwise} & \end{array} \right\}. \tag{8}$$

The actual ingestion rate is then approximated according to the limiting element, following Liebig's minimum law. To do so, we assume fishes have enough food available to meet their minimal needs (S_k). For example, if P is limiting, element-specific ingestion rates, I_k, (g/day) are

$$I_\mathrm{P} = S_\mathrm{P}, \tag{9}$$

$$I_\mathrm{N} = I_\mathrm{P} \frac{D_\mathrm{N}}{D_\mathrm{P}}, \tag{10}$$

$$I_\mathrm{C} = I_\mathrm{P} \frac{D_\mathrm{C}}{D_\mathrm{P}}, \tag{11}$$

where D_k represents element-specific body content percentage of dietary items. Once ingestion rate is estimated, the partitioning of the ingested matter into various pathways (i.e. egestion, excretion and respiration) can be defined.

2.1.3 | Egestion or organic waste production

The rate of organic waste production or egestion rate, W_k (g/day) can be computed using the ingestion rate of each element and element-specific assimilation efficiencies (Schreck & Moyle, 1990):

$$W_k = (1 - a_k)\, I_k. \tag{12}$$

2.1.4 | Total inorganic flux

The rate of total inorganic waste production or flux (i.e. total respiration and excretion) equals the ingestion rate minus body mass growth rate and egestion rate for each element (Barneche & Allen, 2015; Schreck & Moyle, 1990; Sterner & Elser, 2002). If an element is limiting, the individual is likely to consume other elements in excess in order to meet the target for that limiting element. In such cases, it is often assumed that the exceeding 'residual' element will be subject to post-absorptive release via inorganic waste production (i.e. residual flux F_{rk}) to maintain body homeostasis (Anderson et al., 2005). When N or P are limiting, for example, a certain residual amount of C, $F_{r\mathrm{C}}$ remains unutilized. However, if C is limiting instead of N or P, excretion rates F_N and F_P will increase by an overhead residual flux F_{rk}. In the example of C limitation, the residual flux $F_{r\mathrm{C}}$ would equal zero. We can thus quantify the total inorganic flux as follows:

$$F_k = F_{0k} + F_{rk}, \tag{13}$$

where

$$F_{rk} = I_k - G_k - F_{0k} - W_k. \tag{14}$$

2.2 | Application

We validate our modelling approach using data from three reef fish species: the herbivore *Z. scopas* (family Acanthuridae), the omnivore *B. undulatus* (family Balistidae) and the carnivore *E. merra* (family

Serranidae). All parameters were quantified using empirical data augmented with information from the literature when needed (see Supporting Information, Appendix S1). An overview of all parameter estimates is provided in Appendix S2, Table 1.

We ran the model using R (R Core Team, 2019) and Stan (Stan Development Team, 2018). For an easy application of the presented framework, we developed the R package FISHFLUX, which provides a set of user-friendly functions to simulate the model, extract the output variables, and visualize the results (see Appendix S1). Parameter means and standard deviations are provided, and a Monte Carlo method is applied to randomly draw each parameter assuming normal distributions in each iteration. To account for co-variances among parameters, we used the Stan function `multi_normal_rng()`, which samples each parameter under consideration of the co-variance matrix. We included co-variances for body stoichiometry (Q_k), diet stoichiometry (D_k), length–weight parameters (ε and b) and metabolic parameters (α and f_0). These parameters were sampled from their log-transformed multinormal distribution then back-transformed to natural scale. All other parameters were sampled from truncated normal distributions, where the lower and upper bounds are the possible ranges of each respective parameter. For our case study, we used 5,000 iterations. If the standard deviation of a given parameter is unknown (e.g. r, reported on FishBase), the function automatically fills in the standard deviation with a very low value of 10^{-9} in order to keep the respective parameter approximately constant at each iteration of the simulation.

To compare the predictions of ingestion and excretion rates of our model framework with the case where only C-limitation is considered, we simulated ingestion and excretion rates, based only on the minimal supply rate of C, thus where I_C equals S_C. Excretion rates or total inorganic flux rates of N and P are then defined as follows:

$$F_N = S_C \frac{D_N}{D_C} - G_N - W_N, \tag{15}$$

$$F_P = S_C \frac{D_P}{D_C} - G_P - W_P. \tag{16}$$

We compared the predicted excretion rates for N and P with our own independent database of experimental excretion rates. We collected individual fish using barrier nets, dip nets, cast nets, traps, clove oil and hook and line across different reef habitats around Moorea, French Polynesia during austral winter of 2016 and 2017 ($n = 128$). We aimed to collect individuals across the size spectrum present in each species. We immediately transported individuals back to shore in an aerated cooler for excretion experiments (see Appendix S1). Excretion rates were measured within a maximum of 3 hr after capture. The capture and handling of fishes for this project were approved in a protocol from the University of California Santa Barbara's Institutional Animal Care and Use Committee (IACUC #915 2016–2019).

Finally, to illustrate the effect of diet stoichiometry, we simulated the model with varying % of N and P. For this simulation, we used the parameters of *Z. scopas* and ran the simulation for an individual of 10 cm. We kept D_C constant at 20%. The values of D_N and D_P varied around the elemental ratio of S_k. We used colour palettes from the R package FISHUALIZE (Schiettekatte, Brandl, & Casey, 2019).

3 | RESULTS

The application of the developed modelling framework reveals distinct elemental limitations across the three species at different lengths (Figure 2). *Zebrasoma scopas* is limited by either N or P over its full size range, with P being the limiting element early in its ontogeny and N becoming the limiting element after reaching approximately 7 cm TL. Although *B. undulatus* and *E. merra* are also limited by P at an early life stage, they are predominantly limited by C upon maturation.

Our approach demonstrates that defining the limiting element can be critical to predict a species' ingestion rate, which affects all downstream calculations in the model (e.g. excretion rates of N and P) compared to models only considering C limitation (Figure 3). Specifically, assuming C limitation in *Z. scopas* results in a severe underestimation of ingestion and excretion rates (Figure 3a–c). In the omnivore *B. undulatus* and the carnivore *E. merra*, the limiting element has less influence on ingestion rates. Still, without incorporation of P limitation, model predictions may result in negative excretion rates of P for growing individuals of *B. undulatus* and *E. merra*. In the case of *E. merra*, C-only models predict negative P excretion rates

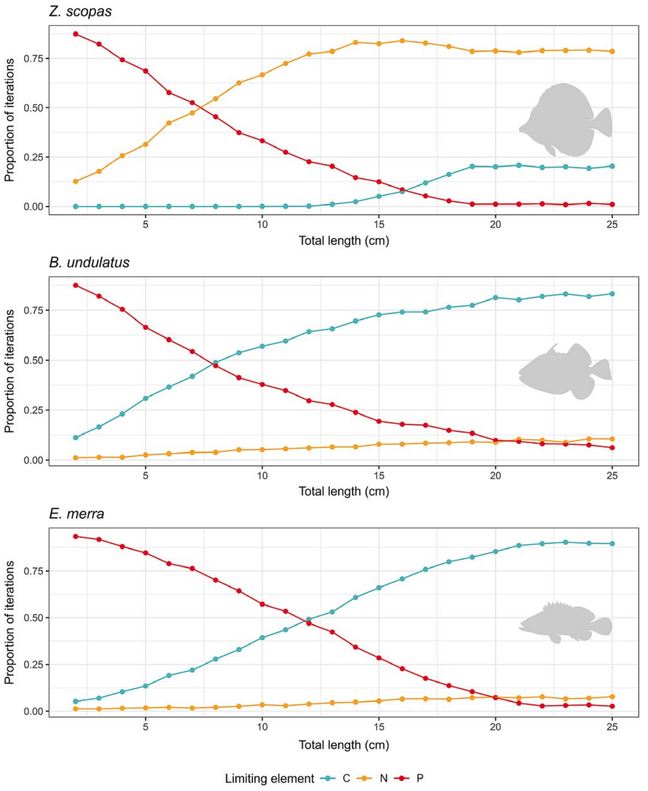

FIGURE 2 Proportion of the simulation iterations that determine C, N and P as the limiting element for *Zebrasoma scopas*, *Balistapus undulatus* and *Epinephelus merra*

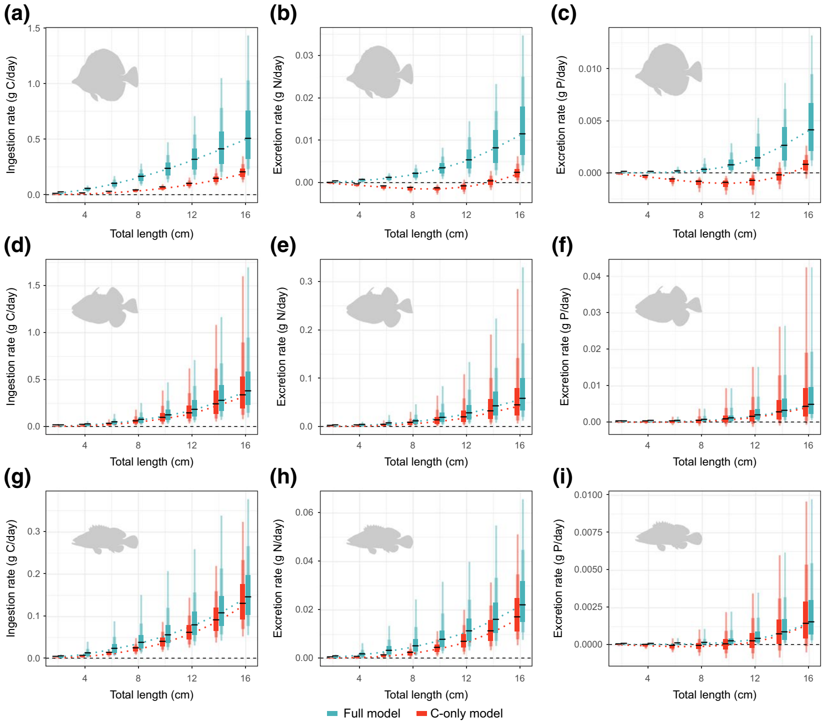

FIGURE 3 Predicted daily ingestion of carbon and excretion rates for the full model, considering nutrient limitation and for a model, only taking into account C-limitation. Horizontal lines show the median values and 95%, 80% and 50% confidence intervals are illustrated respectively in vertical lines. (a) C ingestion rates of *Zebrasoma scopas*, (b) N excretion rates of *Z. scopas*, (c) P excretion rates of *Z. scopas*, (d) C ingestion rates of *Balistapus undulatus*, (e) N excretion rates of *B. undulatus*, (f) P excretion rates of *B. undulatus*, (g) C ingestion rates of *Epinephelus merra*, (h) N excretion rates of *E. merra*, (i) P excretion rates of *E. merra*

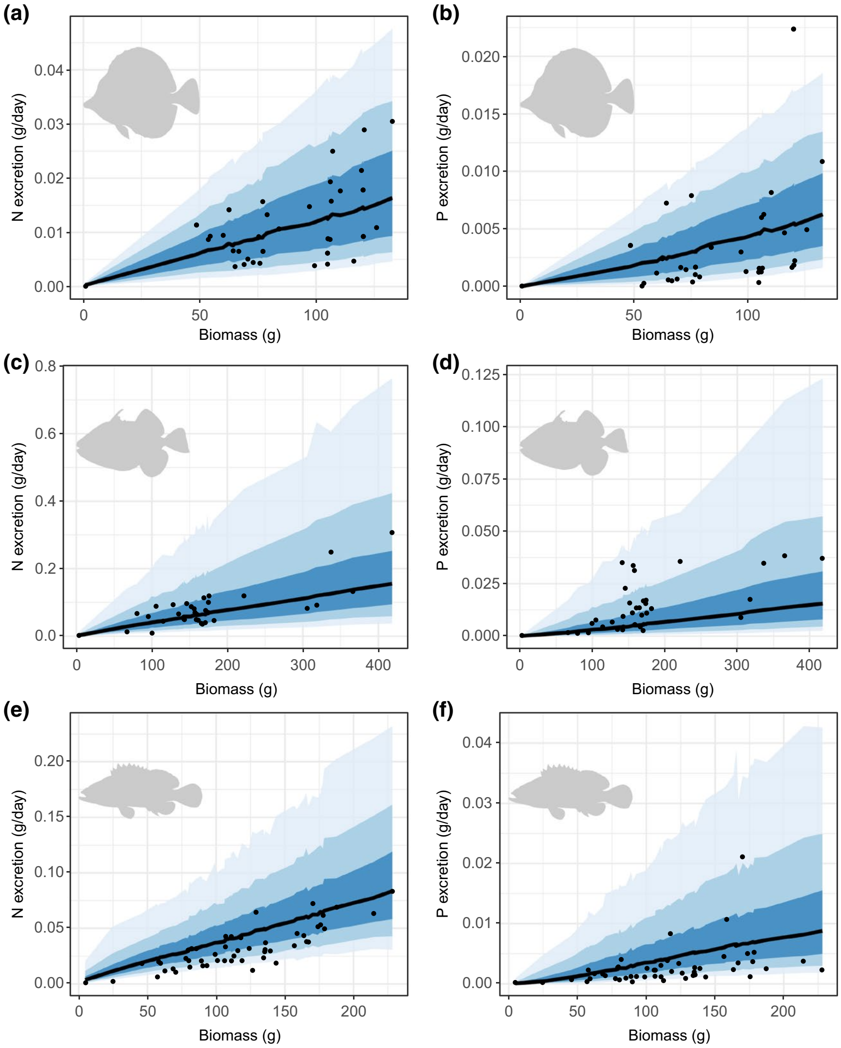

FIGURE 4 Predicted excretion rates for each species of both N and P. The 50%, 80% and 95% confidence intervals are presented around the median. Points show the experimental excretion rates, obtained from an independent database. (a) N excretion rates of *Zebrasoma scopas*, (b) P excretion rates of *Z. scopas*, (c) N excretion rates of *Balistapus undulatus*, (d) P excretion rates of *B. undulatus*, (e) N excretion rates of *Epinephelus merra*, (f) P excretion rates of *E. merra*

for more than half of the simulations under a total length of 10 cm (Figure 3i). Thus, our framework reveals that nutrient limitations and their consequences for ingestion rate estimations are highly specific to the three study species and their ontogenetic stage.

Our model predicts ingestion rates for *Z. scopas*, *B. undulatus* and *E. merra* at 15 cm TL to be 28.2 (11.7–68.4), 12.9 (3.7–56.7) and 14.1 (5.5–40.1), respectively (in mg dry weight per g wet weight of fish per day, median and 95% confidence interval [CI]; see Appendix S2, Table 2). Comparing our predicted excretion rates with empirical data on excretion rates shows that our model adequately predicts excretion rates with almost all experimental data falling inside the predicted 95% confidence interval (Figure 4). For N excretion, 100%, 97% and 94% of the experimental excretion rates are captured by our predictions for *Z. scopas*, *B. undulatus* and *E. merra*, respectively. For P excretion, we adequately predict 93%, 94% and 90% of the experimental excretion rates for the three species, respectively. Predictions for *E. merra* are slightly overestimated compared to experimental excretion rates. Groupers feed infrequently, and their stomachs were often found empty, which may have impacted the measured excretion rates.

Predictions are substantially affected by variability in the stoichiometry of dietary sources. To illustrate how the diet stoichiometry affects limitations by different elements and ingestion and excretion rates, we simulated different scenarios by varying the diet percentages of N and P around the stoichiometry of the minimal supply rate of an individual of *Z. scopas* of 10 cm (Figure 5). When diet stoichiometry differs from this ideal stoichiometry of the minimal supply rate, either C, N or P is the limiting element, which in turn affects all downstream biological processes. For example, when the per cent of P in the diet is low, P is the limiting element (Figure 5a). This leads to an increased ingestion rate (Figure 5b), a minimal excretion rate of P (Figure 5c) and a high excretion rate of N (Figure 5d).

4 | DISCUSSION

Combining stoichiometry and bioenergetic modelling provides a framework to predict elemental fluxes in consumers and their contribution to key biogeochemical cycles. Here, we introduce a model that incorporates the nutrient requirements of fishes alongside their energetic needs to provide accurate predictions of their ingestion, respiration, excretion and egestion rates. With our framework, we confirm the existence of nutrient limitation in fishes, specific to the trophic group and life stage, and its effect on multiple processes. We demonstrate the accuracy and applicability of the model to predict ingestion and excretion rates for three tropical reef fish species, while also reflecting the natural variability of these variables. Our framework provides an accurate tool to predict CNP fluxes in fishes across diverse trophic groups and gauge the role of fish consumers in ecosystems worldwide.

There is a growing consensus that many fishes are limited by nutrients (Benstead et al., 2014; El-Sabaawi et al., 2016; Hood et al., 2005; Moody et al., 2019). Yet, fish growth and maintenance are often assumed to be limited by energy (C) when applying coupled bioenergetic and stoichiometric models (Allgeier et al., 2013; Burkepile et al., 2013; Kraft, 1992; Schindler & Eby, 1997). Our case study confirms that ingestion rates can indeed be determined by N or P limitation rather than C limitation, especially in species with nutrient-poor diets. This finding is expected given the elemental imbalance between the consumer's body and dietary CNP content; however, failing to account for nutrient limitation substantially skews predictions of ingestion rates. For example, assuming only energy limitation for a herbivorous adult *Z. scopas* would result in a greater than twofold underestimation of its ingestion rate and consequently drastic underestimations of excretion and egestion rates. Given the high densities of species with nutrient-poor diets across a variety of ecosystems (e.g. herbivorous and detritivorous species; Hood et al., 2005; Takeuchi, Ochi, Kohda, Sinyinza, & Hori, 2010; Williams & Hatcher, 1983), such underestimates may result in strong

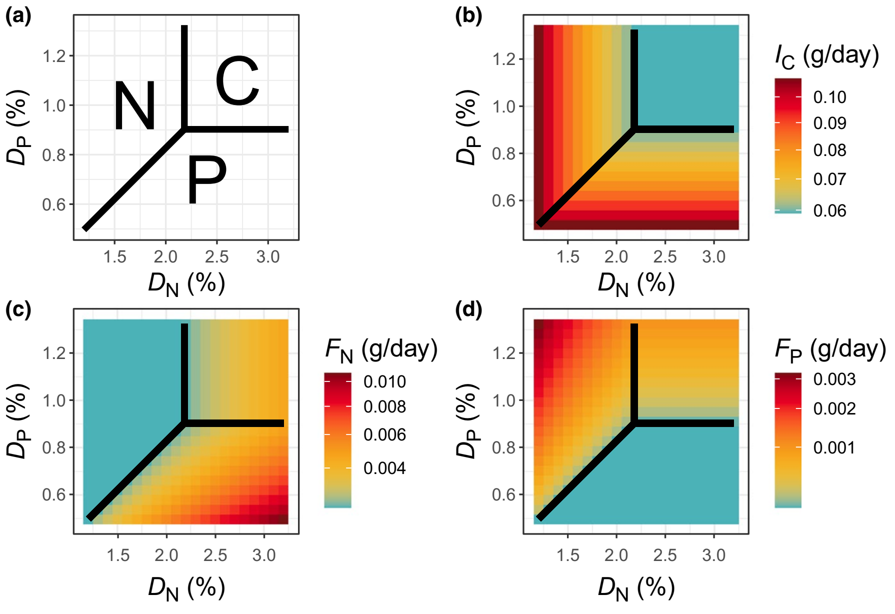

FIGURE 5 Model simulations with varying levels of D_N and D_P. D_C is kept constant. Diet stoichiometry affects the limitation and the rates of multiple processes, such as the ingestion rate and excretion rates. (a) The limiting element is indicated for varying levels of diet stoichiometry (D_N and D_P). Lines indicate where one limiting element switches to another. This is equivalent to the threshold elemental ratio, (b) I_C or ingestion rates of C (g/day), (c) F_N or total inorganic flux of N (g/day), (d) F_P or total inorganic flux of P (g/day)

misconceptions about ecosystem-scale nutrient and energy fluxes. Our model framework provides means for the direct incorporation of varying elemental limitation across species.

The developed model predicts ingestion through the integration of metabolic theory and elemental limitation, thus circumventing the difficult task of measuring ingestion rates in natural populations. Therefore, the first step of our framework focuses on quantifying the minimal supply rate for each element (S_k) and determining the limiting element. This includes both maintenance rates and element-specific growth rates based on the growth trajectory of natural populations. Then, by comparing the stoichiometry of these minimal supply rates with diet stoichiometry, we can determine the limiting element. This approach is inspired by threshold elemental ratio (TER) theory, which predicts the ratio at which growth limitation switches from one element to another (Allen & Gillooly, 2009; Sterner & Elser, 2002; Urabe & Watanabe, 1992). In fishes, it is widely accepted to integrate metabolic rate into the calculation of TERs (Frost et al., 2006). We built on this work to account not only for maintenance requirements of C, but also of N and P. Similar to the energy (C) that is needed to sustain the metabolic rate of fishes in the wild, minimal N and P is needed for cell turnover and maintenance of body composition. The specific turnover rate of P (F_{0Pz}) is lower than the turnover rate of N (F_{0Nz}) because bone cells, which contain the majority of P, degrade slowly compared to other cell types (Manolagas, 2000; Sterner & Elser, 2002). Thus, including minimal requirements for all three elements lowers the TER of C and nutrients of fishes and increases the probability of detecting nutrient limitation.

The inclusion of nutrient limitation ensures that predicted excretion rates (F_P, F_N) are always higher than zero. This is crucial since N and P will always be released at a minimal rate, even when they are limiting (Anderson et al., 2005; Mayor et al., 2011; Sterner & Elser, 2002). Our approach reveals that all three study species are limited by P in their early life. By explicitly including minimal supply rates in our model, we move beyond simply detecting evidence for nutrient limitation (i.e. negative excretion rates; Hood et al., 2005) towards quantifying its effect on vital processes across species and ontogeny. Bone growth, for example, requires substantial amounts of P and is most rapid during early life-stages (Vanni, 2002), and evidence from freshwater ecosystems shows that P can limit fish growth (Benstead et al., 2014; Hood et al., 2005). The ontogenetic variation in elemental limitation presented herein confirms the importance of considering P-limitation for growth when predicting elemental fluxes in fishes.

Beyond the incorporation of nutrient limitation, our model framework provides a way to estimate uncertainty of predictions. Empirically measured excretion rates can considerably vary for similarly sized individuals of the same species (Allgeier, Wenger, Rosemond, Schindler, & Layman, 2015; Francis & Côté, 2018; Whiles, Huryn, Taylor, & Reeve, 2011). Yet, existing models that combine stoichiometry and bioenergetics do not account for this natural variability (e.g. Deslauriers et al., 2017), which hampers our ability to gauge the uncertainty of resulting estimates. With the use of MCMC iterations, the R package FISHFLUX incorporates the distribution of parameters with their means and standard deviations, resulting in realistic credibility intervals of ingestion and excretion rates, although variability in model output does not necessarily reflect natural variability. The utility of this approach is clear when comparing our predictions to reported ingestion rates. For example, *Z. scopas* reportedly ingests 49 mg of dry mass per gram of wet fish weight (Polunin, Harmelin-Vivien, & Galzin, 1995), a value centred within the predicted range of our model (11.7–68.4 at 15 cm TL). Similarly, the ingestion rate of juvenile coral trout, *Plectropomus leopardus*, a predatory species in the same family as *E. merra* (family Serranidae), ranges between 9 and 14 mg of dry mass per gram of wet weight (Sun et al., 2014), which lies within the 95% prediction for *E. merra* from our model (5.5–40.1). Tracing the sensitivity of predictions to uncertainty in specific parameters enables the determination of the main sources of variability that may shift estimates among studies or species.

As all models, our approach relies on several simplifying assumptions. First, our model assumes that fishes maintain homeostasis (Sterner, 1990). Since fishes can have flexible body stoichiometry depending on dietary nutrient content (Benstead et al., 2014; Dalton et al., 2017), this assumption may impose biases when simulating effects of varying diet stoichiometry on elemental fluxes. Yet, empirically measured relationships between nutrient content of body and diet can easily be incorporated into our model simulations, thus ameliorating the effects of this simplification. Second, similar to most stoichiometric mass balance models, our framework is based on Liebig's minimal rule, which states that growth is strictly limited by the element in shortest supply relative to demand. However, there is emerging evidence that consumers may simultaneously be limited by more than one element (Sperfeld, Martin-Creuzburg, & Wacker, 2012). For example, P plays an essential role in fish energy uptake (Xie et al., 2011), and the incorporation of interactive co-limitation into stoichiometric models may further improve predictions of elemental fluxes. Finally, we assume that fishes follow a growth trajectory defined by the VBGC curve, and that there is enough food available in the natural environment to meet the growth requirements for each element. The VBGC is fitted on size-at-age data that are mostly acquired via annual otolith readings. In our model, we use this fitted growth function to estimate daily growth rates for each element through integration with length–weight relationships and body stoichiometry. This does not capture, for instance, seasonal variation of food availability. Other stoichiometric models mostly use gross growth efficiencies (GGEs, i.e. growth/ingestion of the limiting element; e.g. El-Sabaawi et al., 2016; Frost et al., 2006; Guariento et al., 2018; McManamay, Webster, Valett, & Dolloff, 2011; Moody et al., 2019). However, consumer GGEs vary widely, and specific values are poorly understood (McManamay et al., 2011). Furthermore, even if element-specific GGEs are quantified, they may not reflect growth observed in natural populations. Therefore, we suggest that the use of otolith-based growth quantification provides a reasonable alternative to model elemental fluxes of natural fish populations.

Beyond model assumptions, the accuracy of our model naturally relies on the accuracy of each parameter estimate. Yet, parameters are often difficult to obtain. We sought to balance the accuracy of predictions and ease of application. Parameters involving growth, length–weight relationships, metabolism stoichiometry are increasingly accessible for many species due to predictive modelling and open-access databases (e.g. Barneche et al., 2014; Barneche & Allen, 2018; Froese & Pauly, 2018; Froese, Thorson, & Reyes, 2014; Killen et al., 2016; Morais & Bellwood, 2018; Vanni et al., 2017). Yet, there are a number of parameters that are still sparsely quantified and may limit the applicability of our framework. In particular, data on diet stoichiometry and assimilation efficiencies are rare. In our case study, we used assimilation efficiency constants for C, N and P, that are predominantly based on predatory fishes. In reality, assimilation efficiencies can vary substantially, and, in particular, assimilation efficiency of phosphorus is likely correlated with diet quality (Czamanski et al., 2011). Further, N- and P-specific turnover rates are newly introduced parameters and therefore poorly known. As these parameters depend on the cell turnover rates of N- and P-rich tissues (e.g. bone cells for P), we suggest that these parameters may be applicable across species. Nevertheless, further research is needed to gain more insight. While variation in these parameters can impact the model output via the limiting element and ingestion rate, ongoing compilations of databases of poorly known parameters will improve the application of the proposed modelling framework.

In addition, we quantified the activity scope (i.e. field metabolic rate) as the average of maximum metabolic rates (MMR) and standard metabolic rates (SMR) divided by the SMR, assuming that a fish reaches values close to MMR when undertaking activities in the wild (Murchie, Cooke, Danylchuk, & Suski, 2011). In reality, activity scope may vary depending on life history traits and behavior (Killen, Norin, & Halsey, 2017), and field metabolic rates can be elevated with the presence of predators, which in turn can affect nutrient cycling (Dalton, Tracy, Hairston, & Flecker, 2018; Guariento et al., 2018). Refining established techniques, such as bio-telemetry (Norin & Clark, 2016) or otolith chemistry (Chung, Trueman, Godiksen, Holmstrup, & Grønkjær, 2019) may improve estimates of field metabolic rates. Similarly, specific dynamic action (SDA), which is the metabolic rate needed to assimilate food (Hou et al., 2008) depends on the quality and quantity of food (McCue, 2006) and may thus influence ingestion rates, but it is poorly known across most species. Finally, reproduction is not yet incorporated into the model because data on both gonad stoichiometry and reproductive growth are rare. This may underestimate energy and nutrient investment of fishes, thus skewing model predictions. Nonetheless, as new data on reproductive growth, activity scope or SDA become available, these elements can be incorporated in the future.

Despite these limitations, our framework provides new avenues for addressing pressing questions in ecology. Data on the daily actions of fishes are difficult to obtain due to the challenges of conducting research in aquatic environments. Novel techniques such as fish gut content DNA metabarcoding (Casey et al., 2019) or compound-specific stable isotope analyses (Hopkins & Ferguson, 2012) permit improved insights into species-specific ingestion of prey resources. However, no current empirical technique can estimate rates of food ingestion via these linkages across a broad range of species. Combining our model with emerging techniques to quantify species-specific resource use can help us to address long-standing questions. How much prey do top predators consume daily? How do rates of algal consumption differ among herbivorous species? How much production by lower trophic levels is needed to fuel the growth of predatory fisheries species? By providing a tool to answer these questions, our model empowers fundamental and applied researchers to tackle some of the most important questions in fish ecology.

Beyond single species and their pairwise interactions, our model provides means to examine community- and ecosystem-scale dynamics. Specifically, based on simple census data of fish communities, our model can help decompose system-wide fluxes (cf. Allgeier et al., 2014; Burkepile et al., 2013; Francis & Côté, 2018). This is particularly important for open ecosystems in which the dominant sources of energy and nutrients are unclear or variable. For example, on coral reefs, debates persist on the importance of external (i.e. pelagic) subsidies versus internal nutrient cycling (e.g. Brandl, Tornabene, et al., 2019; Morais & Bellwood, 2019). Our model can help estimate how much pelagic or benthic prey is consumed by reef fishes and how these resources are propagated through food webs, which enables researchers to quantify reef functioning (Brandl, Rasher, et al., 2019). Thus, merging what is eaten (i.e. food web assembly) with how much is eaten (i.e. realistic ingestion rates as provided by our model) can significantly augment our understanding of ecosystem functioning, especially in systems where fishes are the dominant consumers.

Finally, given the heavy exploitation of fish communities for global human consumption, our model offers a tool for understanding and predicting the effect of human-driven changes on ecosystem functioning. Yearly, more than 100 million tons of fishes are caught in marine systems worldwide (Cashion et al., 2018). Our model provides a tool to estimate the impact of this disturbance on system-wide biogeochemichal fluxes. In addition, increasing temperatures resulting from climate change can affect primary production in the world's oceans, thus imposing a bottom–up effect on fish communities (Lotze et al., 2019), which are likewise affected by rising temperatures (Pinsky, Eikeset, McCauley, Payne, & Sunday, 2019). Given human-driven alterations in both primary production through climate change and fish community structure through extensive fishing, it is urgent to understand how these changes may impact biogeochemical fluxes. Our model and its implementation provide a path towards rising to this challenge.

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AUTHORS' CONTRIBUTIONS

N.M.D.S. conceived the idea and N.M.D.S., V.P., D.R.B. and S.V. designed methodology; N.M.D.S., J.M.C., S.J.B., A.M., F.M., V.P., K.S.M., J.E.A. and D.E.B. collected the data; N.M.D.S. analysed the data and led the writing of the manuscript. All authors contributed significantly to the drafts and approved the final version for publication.

DATA AVAILABILITY STATEMENT

All data and code to reproduce figures are available online at https://doi.org/10.5281/zenodo.3894509 (Schiettekatte, 2020). The R package FISHFLUX, containing the model can be installed through GitHub: https://github.com/nschiett/fishflux.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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