1   Consumer-driven nutrient recycling of freshwater decapods: linking ecological theories and

2   application in integrated multi-trophic aquaculture

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10   Abstract

11   The Metabolic Theory of Ecology (MET) and the Ecological Stoichiometry Theory (EST) are central

12   and complementary in the consumer-driven recycling conceptual basis. The comprehension of

13   physiological processes of organisms at different levels of organizations is essential to explore and

14   predict nutrient recycling behavior in different scenarios, and to design integrated productive

15   systems that efficiently use the nutrient inputs through an adjusted mass balance. We fed with fish-

16   feed three species of decapods from different families and with aquacultural potential to explore

17   the animal-mediated nutrient dynamic and its applicability in productive systems. We tested

18   whether physiological (body mass, body elemental content), ecological (diet), taxonomic and

19   experimental (time of incubation) variables predicts N and P excretion rates and ratios across and

20   within taxa. We also analysed body mass and body elemental content independently as predictors

21   of N and P excretion of decapods across, among and within taxa. Finally, we verified if body content

22   scales allometrically across and within taxa and if differed among taxa. Body mass and taxonomic

23   identity predicted nutrient excretion rates both across and within taxa. When physiological

24   variables were analysed independently, body size best predicted nutrient mineralization in both

25   scales of analyses. Regarding body elemental content, only body P content scaled negatively with

26   body mass across taxa. Results showed higher N-requirements and lower C:N of prawns than

27   anomurans and crabs. The role of crustaceans as nutrient recyclers depends mainly on the species

28   and body mass, and should be considered to select complementary species that efficiently use

29   feed resources. Prawns need more protein in their feed and might be integrated with fish of higher

30   N-requirements, while crabs and anomurans, with fish of lower N-requirements. Our study

31   contributed to the background of MTE and EST through empirical data obtained from decapods

32   and provided useful information to achieve more efficient aquaculture integration systems.

33

34   KEYWORDS

35   Palaemonidae, Trichodactylidae, Aeglidae, nutrient excretion, aquaculture

36   Introduction

37   Consumers are important nutrient recyclers in aquatic ecosystems as a source or sink for

38   elements such as carbon (C), nitrogen (N) and phosphorus (P) [1,2]. The excretion and

39   egestion of waste products are immediate processes by which animals can be a source of

40   nutrients for primary producers and heterotrophic microorganisms [3]. Animals also constitute

41   nutrient pools, as they grow and reproduce [1,2,4]. As stated by [5], "what animals eat and

42   excrete shapes their role in ecological communities and determines their contribution to the

43   flux of energy and materials in ecosystems". In this sense, two ecological theories are central

44   and complementary in the consumer-driven recycling conceptual basis [6]: the Metabolic

45   Theory of Ecology (MTE) [7] and the Ecological Stoichiometry Theory (EST) [8]. Whereas one

46   emphasizes on energy (MTE), the other does so on matter (EST) [5].

47   The MTE states that the rate at which organisms take up, transform, and expend

48   energy and materials, is the most fundamental biological rate. Organisms are influenced by

49   intrinsic (body size –hereafter, body mass– and stoichiometry of organisms) and extrinsic

50   (temperature) factors, which, in turn, obey to chemical and physical principles [7]. According

51   to the EST, the organismal response to food composition is a useful tool to predict the

52   stoichiometric homeostasis of a given consumer through the selective retain and release

53   (excretion and egestion) of elements like C, N and P. Rates and ratios by which animals

54   recycle nutrients reflect the element imbalance between their bodies and their food [8]. The

55   mass-specific excretion rate of nutrients (i.e. nutrients excreted per unit of body mass per unit

56   time) generally decrease with increasing body mass due to allometric restrictions in

57   metabolism [3,8-10]. A central concept in EST is the Growth Rate Hypothesis (GRH) [11,8],

58   which theorizes that differential allocation of body P content results from differential allocation

59   of P-rich RNA required to protein synthesis during growth. Organisms with high rates of protein

60   synthesis might have lower N:P content. The GRH also predicts that body P-content should

61   be less in small-bodied organisms because these organisms tend to have faster growth rates.

62   [12] proposed a model that suggests that the N:P released by a homeostatic consumer

63   increases with food N:P and decreases with body N:P content. Studies found mixed results in

64   aquatic vertebrates and invertebrates, both supporting and contradicting this mass balance

65   model [13-17]. The variable accuracy of diet and body content as predictors of N:P excretion

66   has been attributed to the flexible homeostasis of organisms [15,16], taxa-specific

67   mechanisms [17], biotic and abiotic factors [18] and resource quality [19]. These mixed results

68   must be due to taxa-specific metabolic processes. [20] found that body mass and taxonomic

69   identity mainly predict excretion rates, but a better understanding of taxa-specific metabolic

70   processes are necessary to predict animal-mediated nutrient recycling. Incubating animals in

71   containers with a known volume of water is a common procedure to measure nutrient

72   mineralization rates [13,21]. However, time and conditions of incubation could also affect rate

73   values and, therefore, experimental design should consider it [22].

74   Variation in body stoichiometry of organisms is hypothesized to be driven by taxon-

75   specific constraints imposed by phylogeny [23-26], differential growth rates and allometry

76   [8,11], structural differences in material allocation [8,27,28], and trophic position [23,25,29]. In

77   the last case, trophic position should predict body composition because organisms should

78   minimize imbalances between the elemental body content and their diet. In this way, predators

79   might have low C:nutrient and high N:P content because they have adapted better to ingest

80   high-nutrient food than lower trophic-positioned organisms [24]. However, high C:nutrient

81   could be found in organisms with C-rich structures, such as the chitin of the exoskeleton, due

82   to differential nutrient allocation [8,27]. In addition, variation in body composition might be due

83   to ontogenetic changes in body stoichiometry and/or P demand associated with faster growth

84   rates [28,30]. Again, taxa-specific metabolic processes constraint the acquisition,

85   incorporation, and release of elements by organisms [31] and, in doing so, scale of analysis

86   may be important to perform any stoichiometric prediction. For example, [25] found family to

87   be the best supported taxonomic level at which body content is conserved in a marine animal

88   community, while [24], in detritus-based communities, and [26], in a global data-set, found that

89   the level of taxonomy that best explains body content depends on the element in question.

90   The role of vertebrates [25,32,33] and invertebrates [31,34,35] as consumer-driven

91   nutrient dynamics in aquatic systems has been extensively studied from different scales of

92   analysis [20,23-26]. Among invertebrates of freshwater systems, decapod crustaceans were

93   also target organisms, either as individuals, species or assemblies, or as agents that regulate

94   the nutrient dynamics of freshwater ecosystems [17,18,28,36-40]. However, this crustacean

95   order is still less evaluated than other taxa [2,28,38] despite the key function they fulfill in

96   ecological process [41-44].

97   In lowland rivers in the Atlantic slope of southern South America, five families of

98   decapods (Sergestidae, Palaemonidae, Parastacidae, Aeglidae and Trichodactylidae)

99   belonging to four suborders (Dendrobranchiata, Caridea, Anomura and Brachyura) comprise

100   the littoral-benthic communities of freshwater systems [45-48]. Despite the phylogenetic

101   distance [49-52], differences in the ventrally folded pleon (carcinization) [53] and lifestyle [47],

102   the trophic habit of these decapods is mostly omnivorous, generalist and opportunistic

103   [46,48,54]. They fulfill key functions in the ecological processes of natural environments by

104   crushing and processing decomposing plant material and by consuming aquatic invertebrates

105   [42,44,54-56]. Although their trophic habits resemble the importance of vegetal and animal

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106   components, they could vary during ontogeny and interspecifically, in addition to extrinsic

107   factors [57-59]. The trophic habit of these organisms is an advantage in captivity conditions

108   because it allows a good acceptance of artificial feed and enables experimental aquaculture

109   research [60, 61].

110   Nowadays, the accumulated information about these decapods is wide. They display

111   different morphological and physiological traits among families, such as foregut types

112   [55,62,63], predation strategies [64-66], enzyme and metabolic activity [61,67,68], and oxygen

113   consumption [69], which certainly influence the resource acquisition, assimilation, and

114   excretion. The holistic overview of this previous information, in addition to an experiment in a

115   laboratory-controlled environment, could be a useful way to understand the taxa-specific

116   differences in stoichiometry and metabolic rates (e.g. excretion) that influence the nutrient

117   dynamic of natural and artificial aquatic systems.

118   In addition, there is an increasing interest in cultivating native species that are adapted

119   to local conditions to conserve local biodiversity [70-72]. The use of native crustaceans in

120   aquaculture systems is growing in South America [73-77]. The potentiality of using them as

121   nutrient recycle organisms in fish production systems is high if we consider their wide trophic

122   habits, the great acceptance of artificial feed, and the multiple uses of this by-product to add

123   revenue to the production [60,78,79]. This approach is used to deal with the environmental

124   burden of intensive farming and is coupled with the practice of integrated multitrophic

125   aquaculture (IMTA), which consists in co-cultivating organisms of different trophic levels in the

126   same system selected for their functions and complementary ecosystem services, connected

127   by the transfer of matter and energy through water [80]. The low diversity of these artificial

128   aquatic systems implies a strong influence of few taxa on the nutrient turnover [14,81] and

129   emphasizes the importance of studying species with aquacultural potential and interest.

130   Coupling experimental aquaculture research with ecological theories is an interesting

131   way to explore the animal-mediated nutrient dynamic and its applicability in productive

132   systems. Here we test how physiological (body mass, body elemental content), ecological

133   (diet), taxonomic and experimental variables explain nutrient recycling at order (across taxa)

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134   and at family/species (among and within taxa) levels, in order to discuss them from an

135   ecological and applied point of view. We used three species of decapods from different

136   suborders and families, with antecedents about trophic and digestive ecology and with

137   aquacultural potential use. We are interested in understanding their potential nutrient recycle

138   role in natural and artificial systems, such as in an IMTA. We performed an experimental

139   research using two different commercial feedstuffs elaborated for detritivorous and

140   omnivorous fish, and we analysed the excretion response variables (mass-specific excretion

141   rates of N, P and associated ratios) in two different incubation's period. We also evaluated the

142   body elemental content of C, N, P and associated ratios to relate these results with

143   antecedents about trophic habits, carcinization and ontogeny.

144   We asked three main questions **(Q)**. **Q1:** Which of the physiological (body mass, body

145   elemental content), ecological (diet), taxonomic and experimental (time of incubation)

146   variables best predicts N and P excretion rates and ratios across and within taxa? We

147   hypothesized that: **H1-** Physiological and taxonomic variables best explain the variation in N

148   and P excretion rates. **Q2: a)** Could physiological variables (body mass, body elemental

149   content), if analysed independently, predict N and P excretion rates and ratio of decapods,

150   according to MTE and EST? Are these allometric (excretion rate vs. body mass) and

151   stoichiometric (excretion rate vs. body elemental content) variations observed across and

152   within taxa? **b)** Are allometric and stoichiometric variations different among taxa? With respect

153   to these questions, we formulated three hypotheses: **H2-** N:P excretion rates decrease with

154   increasing N:P body content, as postulated by Sterner (1990), both across and within taxa.

155   **H3-** N and P excretion rates scale allometrically across and within taxa. **H4-** There are

156   allometric and stoichiometric differences among taxa. **Q3: a)** Do body content scales

157   allometrically across and within taxa? **b)** Are body elemental content and allometric variations

158   different among taxa? We hypothesized that: **H5-** Body P content is negatively related to body

159   mass across and within taxa, according to GRH. **H6-** Body C:N content increases in carcinized

160   crustaceans and decreases, together with N:P, in lower trophic positioned species.

161

Material and methods

Studied organisms

Decapod species used in this experiment represent three different families of neotropical decapod crustaceans. *Macrobrachium borellii* is a prawn of the Palaemonidae family, with wide distribution in La Plata Basin of northern Argentina, Paraguay and southern Brazil [82, 83]. Its natural diet exhibits a significant presence of animal items such as dipterans and oligochaeta larvae, and a low importance of vegetal remains and algae [47, 54, 56]. It is a species characterized by moving in the water column and towards the littoral vegetation [62, 84]. *Aegla uruguayana* belongs to the Aeglidae family, which has a unique genus endemic to southern South America. This species presents benthic habits, typically sheltered at the bottom and under rocks of current rivers and streams [82,85] and displays strong swimming habits [86,87]. Its natural diet is composed mainly of vegetal remains and diatoms with low importance of animal items [54,55]. *Trichodactylus borellianus* belongs to the neotropical family of freshwater crabs, Trichodactylidae, with broad distribution in South America (from 0° to 35° S) [88]. This crab has a close relationship with the floating aquatic vegetation and exhibits little mobility [48,89]. Its natural diet is characterized by vegetal remains, algae and animal items such as oligochaetes and insect larvae [90,56]. Both prawns and crabs form abundant populations associated with the aquatic macrophytes of the floodplain littoral zone [46,89,91].

Crustaceans sampling and laboratory maintenance

Specimens of crustaceans of varied sizes were manually collected from the environment with the aid of a hand net (500 μm mesh size) during the austral late spring (November and December 2018). *Macrobrachium borellii* and *T. borellianus* were captured from the "Ubajay" stream (31°33'43.45''S, 60°30'58.73''W), Santa Fe (Argentina), among the aquatic vegetation

187   at the shoreline of water bodies. *Aegla uruguayana* were captured from "El Espinillo" stream

188   (31°47'09.16''S, 60°18'57.46''W), Entre Ríos (Argentina), through the removal of stones at the

189   bottom of the stream and placing the hand net against the current. Crustaceans were

190   translated to laboratory in plastic containers, where they were acclimated gradually (at least

191   two weeks) to the experimental conditions (temperature - 24 ± 1 °C; natural photoperiod –

192   dawn and dusk around 05:00 and 20:00, respectively; conductivity– 250 ± 10 µS) in aquaria

193   with dechlorinated and aerated tap water, with rocks and PVC tubes as refugees. During this

194   period, crustaceans were fed *ad libitum* with the same fish feed used in the experiments. Two

195   extruded commercial feeds (Garay SRL) were used. They were elaborated for the nutrition of

196   omnivorous (OF) and detritivorous fish (DF) (e.g. pacú - *Piaractus mesopotamicus* and sábalo

197   - *Prochilodus lineatus*) (Table 1). Both feeds were ground in a mortar and passed through

198   sieves of 1000-µm-diameter mesh to attain a size that facilitates the ingestion by crustaceans.

199   Then, feeds were stored in glass bottles at 5°C. Every 48 hours, feces and food remains were

200   removed by siphoning and the discharged water was supplemented. Every day pH,

201   conductivity, dissolved solids and temperature were measured with a waterproof tester (Hanna

202   HI98129, Romania), and dissolved oxygen with an oximeter to verify water quality (YSI Proodo

203   SKU626281, USA).

204

205   Table 1. Proximal and elemental (C, N, P) composition of detritivorous (DF) and omnivorous

206   (OF) fish feeds used in the experiments.

Ingredients	OF (%)	DF (%)
Proximal composition (g/100 g dry basis)[1]		
Protein	27	30.68
Lipids	3.87	5.00
Humidity	---	3.82
Crude Fiber	3.53	12.24

Ash	4.28	8.37
P total	0.80	1.24
Elemental composition (g/100 g dry basis)		
C	42.4±0.3	41.4±0.1
N	3.8±0.1	4.9±0.2
P	1.1±0.2	1.6±0.2

207 [1] Garay SRL (Recreo, Argentina) – nutritional values analyzed by the manufacturer. --- not informed by the

208 manufacturer

209

Experimental design and procedures

211 The feeding experiment was carried out using specimens of each species with variable body

212 mass and control for each treatment. Only *A. uruguayana* had a reduced number of specimens

213 in the experiment due to the limited wild caught species. Feeds used were commercial

214 formulations designed for the nutrition of fishes with omnivorous and detritivorous feeding

215 habits (Garay SRL, Argentina).

216 After the acclimation period, organisms were transferred to individual plastic recipients

217 of 1 liter, randomly and interspecified arranged, where they were left for 24 hours without food.

218 In total, 66 individuals were used (24 of *M. borellii*, 18 of *A. uruguayana* and 24 of *T.*

219 *borellianus*) plus six controls without specimens. Each recipient was provided with shelter (a

220 small rock previously washed and chlorinated), artificial aeration and covered with a half

221 shadow to reduce stress and prevent escapes. After the fast period, fish feed was offered *ad*

222 *libitum* to the corresponding treatment and left for 90 minutes. Then, each organism was

223 removed from the plastic recipients, washed with distilled water, and transferred to glass

224 bottles with 150 ml of filtered (MG-F 0.7 µm, Munktell Filter-Sweden) and dechlorinated tap

225 water. Then, 15 ml of water samples were taken from each recipient at 30 and 60 minutes

226 using a micropipette (5000 µl), and they were conserved at -20°C until the analytical

227 determination of excreted nutrients was performed. The incubation time of 60 minutes was

228   previously considered adequate because excretion rates rapidly decrease after organisms

229   stop the feed ingestion [92]. However, water samples were taken in each recipient at 30 and

230   60 minutes to verify if these incubation time lapses (also mentioned as time) represent a

231   variation in nutrient excretion rates and associated ratios.

232   At the end of the experiment, crustaceans were kept without feed for 24 hours to

233   eliminate the gut content. Then, animals were anesthetized in cold water and frozen.

234   Subsequently, individuals were oven dried at 50 °C to constant weight, and weighted (± 1 µg)

235   to determine dry body mass. The dried body of each individual was pulverized and

236   homogenized in a mortar, and elementally analysed (C, N and P). This study adheres to the

237   ethical standards of [93], and ethical and legal approval was obtained prior to the start of the

238   study by the committee of ethics and safety in experimental work of CONICET (CCT, Santa

239   Fe).

240

Analytical and elemental analysis

242   Water samples from each treatment were analysed for inorganic forms of N and P, ammonium

243   (NH_4-N) and orthophosphate (P-PO_4), respectively. NH_4-N was quantified through the

244   indophenol blue method [94], and P-PO_4 through the ascorbic acid method [95]. To determine

245   the stoichiometric proportion of carbon (C) and nitrogen (N) of crustaceans and feeds, two

246   subsamples of each sample were elementally analysed in a CHN628 Series Elemental

247   Determinators (LECO ®). For total phosphorus (P) analysis, dry crustaceans' bodies or ground

248   powder feed were weighted (± 1 µg) and combusted in a muffle furnace at 550°C for a

249   minimum of 2 hours. Then, the mass of ash was weighted and acid-digested with 25 ml of HCl

250   1N during 15-20 minutes in a heating plate. The digested solution was brought to 100 ml with

251   distilled water [96] and analysed using the ascorbic-acid method [95]. C, N and P of feed and

252   crustaceans' bodies were expressed as g/100g at a dry weight basis (dw) and ratios C:N, C:P

253   and N:P were calculated using molar values.

254   The amount of ammonium and orthophosphate obtained in each excretion chamber

255   was corrected by subtracting the average of nutrient concentration obtained in the control

256   replicates (chambers without crustaceans) from the value obtained in crustaceans' excretion

257   chambers after 30 and 60 minutes. Then, results were divided by the body mass (mg of dry

258   weight) and by the time of incubation (0.5 or 1 hour). Mass-specific NH_4-N, P-PO_4 and NH_4-

259   N:P-PO_4 excretion rates were expressed throughout the text as N, P and N:P excretion or

260   mineralization.

261

262 Data analysis

263   The differences of elemental composition between two extruded commercial feeds for OF and

264   DF were analyzed through Mann-Whitney-Wilcoxon Test with R software version 3.6.3 [97].

265 Nutrient excretion rates

266   **Q1-** Mixed-effects models with individuals as a random variable (lmerTest R package) [98]

267   were used to analyse the effect of variables on the N, P and N:P excretion across and within

268   taxa. The 'Drop1' function was run on the mixed-effects models to select the variables that

269   best explain nutrient excretion rates and ratios variations. This function computes likelihood

270   ratio test statistics and p-values for all single terms, fits those models and computes a table of

271   the changes in fit. Only relevant interactions then compose the statistical table. The 'single

272   term deletion' is useful for model selection and tests of marginal terms [98].

273   Mixed models were chosen, starting with the interaction effects of species variable

274   (also named as taxonomic identity) (only for across taxa analysis), time and type of feed as

275   factors, and body mass and body elemental content as covariates. Individuals were included

276   as random effects to assess how time affects nutrient rates. If the term time was statistically

277   significant, the data with higher nutrient excretion rates will be selected to run the posterior

278   regressions and analysis of covariance (ANCOVA).

279   **Q2- a)** After running mixed models, allometric relationships across and within taxa were

280   analysed between N, P and N:P excretion for 30 or 60 minutes by regressing these parameters

281   as a function of species body mass. Also, stoichiometric relationships were quantified, across

282   and within taxa, regressing nutrient excretion rates and ratios as a function of body N, P and

283   N:P content. **b)** Allometric and stoichiometric trends among taxa were compared using

284   ANCOVA analysis, including nutrient excretion rates and ratios (data of 30 or 60 minutes

285   according to lmer results) as dependent variables with body mass and body elemental content

286   (N, P and N:P) as covariates, respectively. Statistical differences among taxa were determined

287   using Bonferroni adjustment for multiple testing corrections with the function emmeans_test

288   of emmeans package [99] (Lenth et al., 2021).

Body elemental content

290   **Q3- a)** Allometric relationships across and within taxa were analysed between body N, P, C

291   content and associated ratios by regressing these parameters as a function of species body

292   mass. **b)** The body content of nutrients and ratios were compared among taxa through

293   ANCOVA and using body mass as covariate. Statistical differences among species were

294   determined using Bonferroni adjustment for multiple testing corrections with the function

295   emmeans_test of emmeans package [99].

296   All analyses of nutrient excretion rates and body elemental content were conducted

297   with R software version 3.6.3 [97] and all variables used were $\log_{10}$-transformed to standardize

298   variance among species.

299   Finally, the relationship between $\log_{10}$-transformed physiological variables (body mass

300   and body elemental content) and nutrient excretion was shown through Principal Component

301   Analysis (PCA), based on a correlation matrix, as a conclusion and visualization of the main

302   results. This analysis included nutrient excretion rates and ratios of the selected time (30 or

303   60 minutes), body elemental contents (single elements and ratios), and body mass,

304   considering the taxonomic identity of each species.

305

Results

307 The elemental composition analysis determined that DF presented a slightly higher amount of

308 nitrogen (4.9 ± 0.2%) and phosphorus (1.6 ± 0.2%) compared to the OF (3.8 ± 0.1% and 1.1

309 ± 0.2%, respectively), but these differences were not significant (W= 0, *p*= 0.2 and *p*= 0.1,

310 respectively). Regarding the percentage amount of carbon, both feeds were similar (W: 6; *p*=

311 0.2) (Table 1). Mean body masses of decapods used in the experiments were: *M. borellii*

312 (351.0 ± 82.1 mg dw), *A. uruguayana* (554.2 ± 536.8 mg dw) and *T. borellianus* (110.8 ± 50.7

313 mg dw), with ranges of [44.3 - 377.6 mg dw], [8.2 – 1900.1 mg dw] and [26.7 – 220.9 mg dw],

314 respectively.

Nutrient excretion

316 **Q1-** Across taxa analysis identified species (taxonomic identity) and body mass as the

317 variables that best explained N and P excretion, whereas only taxonomic identity explained

318 variations in N:P excretion (Table 2). The type of diet and body N, P, and N:P content did not

319 affect variations of nutrient excretion rates and ratio (Table 2). Moreover, time and its

320 interaction with the taxonomic identity influenced the variation of N excretion (Table 2).

321 Excretion levels of N were higher at 30 minutes (0.9574 ± 1.1097 µg N mg dw^{-1}.hr^{-1}) than at

322 60 minutes (0.7279 ± 0.7373 µg N mg dw^{-1}.hr^{-1}). According to these results, posterior

323 regressions and ANCOVAs analyses were made using the data obtained at 30 minutes.

324

325 Table 2. Results of the variables selected by 'Drop1' function to explain the nutrients excretion

326 rates and ratios, ran on the mixed-effects model across taxa.

	F-value	p-value
N excretion		
Time: Species	5.4434	0.0096 **

Time: Diet	1.1026	0.3020
Species: Diet	1.2891	0.2925
Time	8.2662	0.005643 **
Species	42.985	5.241×10^{-12} ***
Diet	1.4157	0.2392
Body mass	19.7381	0.0001 ***
Body N content	0.3992	0.5330
P excretion		
Time: Species	1.1041	0.3408
Time: Diet	0.6410	0.4282
Species: Diet	0.4949	0.6129
Time	1.6150	0.2096
Species	17.7731	1.006×10^{-06} ***
Body mass	5.4282	0.0237 *
Body P content	0.1090	0.7430
N:P excretion		
Time: Species	0.6922	0.5108
Time: Diet	0.6355	0.4336
Species: Diet	1.8923	0.1608
Time	0.1359	0.7139
Species	39.0418	2.226×10^{-11} ***
Body mass	0.0236	0.8793
Body N:P content	0.0814	0.7781

327   Statistically significant differences *$p < 0.05$, **$p < 0.005$, ***$p < 0.001$.

328

329   According to taxa analysis, the variables that most affect N excretion of the prawn *M.*

330   *borellii* were body mass and body N content (Table 3), while any variable explained P excretion

331   variations in this species (Table 3). Body mass explained the N and P excretion variations of

14

332   *A. uruguayana* (Table 3)*. Moreover, time influenced the N excretion of this species (Table 3),

333   with values higher at 30 minutes (0.9553 ± 1.6341 µg N mg d $w^{-1}.hr^{-1}$) than at 60 minutes

334   (0.3128 ± 0.3542 µg N mg d $w^{-1}.hr^{-1}$). *T. borellianus* crab did not show a significant relationship

335   between N and P excretion with any analysed variables (Table 3). Additionally, any variable

336   explained N:P excretion variations in any species (Table 3).

337

338   Table 3. Results of the variables selected by 'Drop1' function to explain the nutrients excretion

339   rates and ratios, run on the mixed-effects model within taxa.

	F-value	p-value
Excretion N		
M. borellii		
Time: Diet	1.3056	0.2798
Time	0.3264	0.5792
Diet	0.0502	0.8282
Body mass	8.9670	0.0172 *
Body N content	6.2851	0.0365 *
A. uruguayana		
Time: Diet	3.2176	0.1030
Time	19.7189	3×10^{-4} ***
Diet	1.3291	0.2671
Body mass	9.1818	0.0163 *
Body N content	0.1982	0.6679
T. borellianus		
Time: Diet	0.0486	0.8311
Time	0.1212	0.7320
Diet	1.4188	0.2521
Body mass	1.6631	0.2447

15

Body N content	1.3292	0.2928
Excretion P		
M. borellii		
Time: Diet	0.3710	0.5504
Time	4.0167	0.0596
Diet	1.1349	0.2999
Body mass	0.0087	0.9267
Body P content	0.0596	0.8101
A. uruguayana		
Time: Diet	2.1144	0.1715
Time	1.8092	0.1974
Diet	0.7377	0.4031
Body mass	15.3083	0.0020 **
Body P content	0.0175	0.8969
T. borellianus		
Time: Diet	2.5337	0.1366
Time	0.0000	0.9947
Diet	1.4253	0.2401
Body mass	0.7611	0.4017
Body P content	0.2193	0.6487
Excretion N:P		
M. borellii		
Time: Diet	1.5418	0.2425
Time	0.4891	0.4938
Diet	0.0328	0.8585
Body mass	0.6743	0.4351
Body N:P content	0.1420	0.7168

A. uruguayana		
Time: Diet	2.7509	0.1544
Time	0.3512	0.5604
Diet	1.5098	0.2342
Body mass	0.0097	0.9254
Body N:P content	0.9287	0.3637
T. borellianus		
Time: Diet	0.4942	0.5129
Time	0.1916	0.6768
Diet	4.1843	0.1165
Body mass	0.5910	0.4862
Body N:P content	0.1285	0.7400

340 Statistically significant differences *$p < 0.05$, **$p < 0.005$, ***$p < 0.001$.

341

342 **Q2- a)** According to regressions performed at 30 minutes and across taxa, N and P

343 excretion rates decreased significantly with increasing decapods body mass. Decapods' body

344 mass explained more variation in N (45% of variance) than in P excretion (13% of variance)

345 (Fig 1a, b). N:P excretion did not show significant differences with crustaceans' body mass

346 (Fig 1c). The stoichiometric relationship between N excretion and body N content was

347 significant and positive, with an explanation of 36% (Fig 1d), whereas P excretion did not show

348 significant differences with body P content (Fig 1e). N:P excretion decreased significantly with

349 increasing body N:P content, with a low explanation (18% of variance) (Fig 1f).

350 Regarding regressions carried out at 30 minutes within taxa, N excretion of prawn *M.*

351 *borellii* decreased significantly with increasing prawn body mass although it showed a low

352 allometric relationship (Table 4) (Fig 1a). This prawn did not show a significantly linear

353 relationship with P excretion and body mass (Table 4) (Fig 1b). *A. uruguayana* aeglid showed

354 a significant and negative allometric relationship between N and P excretion and body mass,

355 and this relationship was stronger in N than in P excretion (Table 4) (Fig 1a, b). *T. borellianus*

17

356 crab showed a significant linear relationship between N excretion and body mass, but this was

357 on the edge of significance (Table 4) (Fig 1a). This crab showed no significant linear

358 relationship between P excretion and body mass (Table 4) (Fig 1b). According to N:P

359 excretion, this variable did not show significant linear relationship with body mass in any

360 species of decapods (Table 4) (Fig 1c). On the other hand, there was no significant linear

361 stoichiometric relationship between nutrient excretion and body elemental content in any

362 species of decapods (Table 4) (Fig 1d, e, f).

363

364 Fig 1. Linear regressions across and within taxa of excretion rates of N, P and N:P as a

365 function of invertebrate body mass (a, b, c) and body elemental content (d, e, f). The equation,

366 R-squared and p-value belong to the across taxa linear regressions. *Macrobrachium borellii*

367 (black circle), *Aegla uruguayana* (dark gray square), *Trichodactylus borellianus* (light gray

368 triangle).

369

370 Table 4. Within taxa linear regressions of nutrient excretion rates (N, P) and ratio (N:P) as a

371 function of crustaceans' body mass.

	Slope (b)	R^2	p-value
N excretion *vs* body mass			
M. borellii	**-0.5887**	**0.3430**	0.0026 **
A. uruguayana	**-0.7518**	**0.8544**	4.25×10^{-08} ***
T. borellianus	**-0.5628**	**0.2203**	0.0426 *
P excretion *vs* body mass			
M. borellii	-0.3978	0.1288	0.1100
A. uruguayana	**-0.6783**	**0.6179**	5×10^{-4}***
T. borellianus	-0.9372	0.0547	0.3570
N:P excretion *vs* body mass			
M. borellii	0.0395	0.0073	0.6920

A. uruguayana	-0.1600	0.0656	0.3568
T. borellianus	0.7526	0.0299	0.5340
N excretion *vs* body N			
M. borellii	-5.1430	0.2069	0.1374
A. uruguayana	2.0270	0.0261	0.6155
T. borellianus	4.5670	0.3190	0.0889
P excretion *vs* body P			
M. borellii	-3.0430	0.0497	0.5639
A. uruguayana	-1.4063	0.0116	0.7129
T. borellianus	-2.1786	0.0112	0.6954
N:P excretion *vs* body N:P			
M. borellii	-0.1773	0.0107	0.7485
A. uruguayana	-2.1400	0.1334	0.2995
T. borellianus	0.1442	0.0004	0.9640

372 Statistically significant linear relationships between species body mass and the respective response variable *$p <$

373 0.05, **$p <$ 0.005, ***$p <$ 0.001.

374

375 **b)** Among taxa, it was observed a significant individual effect of taxonomic identity and

376 body mass (and not interactive effect) on N (ANCOVA, $p = 2.79 \times 10^{-12}$ for species effects and

377 $p = 2.84 \times 10^{-14}$ for body mass effect) and P (ANCOVA, $p = 0.0004$ for species effects and $p =$

378 0.0002 for body mass effect) excretion. N:P excretion showed a significant difference only

379 between taxonomic identity and not as regards body mass (ANCOVA, $p = 8.29 \times 10^{-10}$ and $p =$

380 0.640).

381 When body elemental content was used as a covariate, it was observed a significant

382 individual effect of taxonomic identity and body N content (and not an interactive effect) on N

383 excretion (ANCOVA, $p = 0.0014$ and $p = 0.0003$, respectively). Whereas in P and N:P excretion,

384 there was only a significant individual effect of taxonomic identity (ANCOVA, $p = 0.0094$ and

385 $p = 1.03 \times 10^{-6}$) and not of body elemental content.

19

386   Regarding *post hoc* tests, N excretion was statistically different among all species

387   (Bonferroni *post hoc*, *p*< 0.001) (Fig 2). *M. borellii* prawn showed the highest average value of

388   N excretion, *A. uruguayana* showed an intermediate one, and *T. borellianus* the lowest one

389   (Fig 2a). P excretion was statistically different among *M. borellii* prawn and the other species

390   (Bonferroni *post hoc*, *p*< 0.001) (Fig 2b). This species showed lower average value of P

391   excretion than the other species (Fig 2b). The N:P excretion differed significantly among all

392   species (Bonferroni *post hoc*, *p*< 0.001) (Fig 2c). *M. borellii* prawn showed the lowest average

393   value of N:P excretion, *A. uruguayana* showed the highest one, and *T. borellianus* an

394   intermediate one (Fig 2c).

395

396   Fig 2. Box plots of excretion rates of N, P and N:P of each decapod species (a, b, c). The top,

397   bottom, and line through the middle of the boxes correspond to the 75th, 25th, and 50th

398   (median) percentile. The whiskers extended from the 10th percentile to the 90th percentile.

399   Crosses indicate the median values. Different letters above bars indicate significant

400   differences among taxa (*p* < 0.05).

401

Body elemental content

403   **Q3- a)** Regressions across taxa showed a significant negative allometric relation between

404   body P content and body mass (Fig 3b). Whereas the other body content elements and ratios

405   did not show a significant relation with body mass (Fig 3a, c, d, e, f). As regards taxa

406   regressions, only *T. borellianus* crab showed a significant negative allometric relationship

407   between body N content and body mass (Table 5) (Fig 3a). Variations in body P and C content

408   did not show significant linear relationship with body mass in any species of decapods (Table

409   5) (Fig 3b, c). Only *M. borellii* prawn and *T. borellianus* crab showed a significant positive linear

410   relationship between body C:N content and body mass (Table 5) (Fig 3e).

411

412   Fig 3. Linear regressions across and within taxa of body content of N, P, C and N: P, C:N, C:P

413   as a function of invertebrate body mass. *Macrobrachium borellii* (black circle), *Aegla*

414   *uruguayana* (dark gray square), *Trichodactylus borellianus* (light gray triangle).

415

416   Table 5. Within taxa linear regressions of body elemental content (N, P, C, N:P, C:N, C:P) as

417   a function of decapods' body mass.

	Slope	R^2	p-value
Body N			
M. borellii	-0.0311	0.1371	0.2520
A. uruguayana	-0.0232	0.1268	0.3460
T. borellianus	**-0.0957**	**0.3930**	0.0290 **
Body P			
M. borellii	0.0133	0.0059	0.7256
A. uruguayana	-0.0115	0.0195	0.6060
T. borellianus	-0.0210	0.0041	0.8117
Body C			
M. borellii	0.0265	0.1620	0.1945
A. uruguayana	0.0009	0.0001	0.9705
T. borellianus	-0.0127	0.0067	0.7990
Body N:P			
M. borellii	-0.0138	0.0038	0.8488
A. uruguayana	-0.0121	0.0180	0.6776
T. borellianus	-0.1286	0.0839	0.4865
Body C:N			
M. borellii	**0.0576**	**0.5598**	0.0051**
A. uruguayana	0.0241	0.0955	0.3284
T. borellianus	**0.0845**	**0.4889**	0.0114 *

Body C:P			
M. borellii	0.0437	0.0503	0.4831
A. uruguayana	0.0119	0.0263	0.6139
T. borellianus	-0.0899	0.0413	0.6293

418   Statistically significant linear relationships between species body mass and the respective response variable *$p <$

419   0.05, **$p <$ 0.005, ***$p <$ 0.001.

420

421   **b)** Among taxa, it was observed a significant individual effect of the taxonomic identity

422   on body N, P, C, N:P, C:N and C:P contents (ANCOVA, $p= 2 \times 10^{-16}$, $p= 9.56 \times 10^{-13}$, $p= 6.84 \times 10^{-5}$,

423    $p= 3.95 \times 10^{-10}$, $p= 1.05 \times 10^{-14}$ and $p= 3.71 \times 10^{-7}$, respectively) and significant individual effect

424   of body mass on body N (ANCOVA, $p= 0.0001$), body P (ANCOVA, $p= 3.54 \times 10^{-6}$) and body

425   C:N contents (ANCOVA, $p= 0.0052$). According to *post hoc* interspecific analysis, body N and

426   C contents were statistically different among *M. borellii* prawn (with highest average values)

427   and the other species (Bonferroni *post hoc*, $p< 0.001$) (Fig 4a, c). This prawn also showed

428   significant differences in body C:N content between the other species (Bonferroni *post hoc*,

429    $p< 0.001$), but with lowest average values (Fig 4e). Percentage of body P and N:P contents

430   were statistically different among all species (Bonferroni *post hoc*, $p< 0.001$) (Fig 4b, d). *T.*

431   *borellianus* crab showed the highest average value of body P content and *A. uruguayana*

432   aeglid, the lowest one (Fig 4b). Moreover, *M. borellii* prawn showed the highest value of body

433   N:P content and *T. borellianus* crab, the lowest one (Fig 4d). Finally, *T. borellianus* crab

434   showed statistically differences in body C:P content in comparison to the other species

435   (Bonferroni *post hoc*, $p< 0.001$), with the lowest average values (Fig 4f).

436

437   Fig 4. Box plots of body content of N, P, C and N:P, C:N, C:P of each decapod species. The

438   top, bottom, and line through the middle of the boxes correspond to the 75th, 25th, and 50th

439   (median) percentile, respectively. The whiskers extended from the 10th percentile to the 90th

440   percentile. Crosses indicate the median values. Different letters above bars indicate significant

441   differences among taxa ($p < 0.05$).

442

443   The first two components of PCA explained the 39.38% (Component 1) and 22.13%

444   (Component 2), showing variations among species (Fig 5). Body N, C, N:P and C:N contents

445   characterised species' variations with high correlation values in Component 1 (Table 6). Body

446   N, C, and N:P contents were higher in *M. borellii* prawn and lower in the other crustaceans,

447   while body C:N followed the opposite trend. Body P content showed high correlation values in

448   Component 2 (Table 6), being higher in *T. borellianus* and lower in *A. uruguayana* (Fig 5). The

449   body C:P content showed similar values of correlation in both components, and was low in *T.*

450   *borellianus*. The nutrient excretions and ratio presented low values of correlation in both

451   components. However, it was observed that N excretion was higher in *M. borellii* and lower in

452   *A. uruguayana.* Besides, this variable increased with body N content and decreased with body

453   mass. *T. borellianus* crab exhibited higher P excretion and body P content, which decreased

454   with body mass. The N:P excretion increased in *A. uruguayana* and with body mass. Body

455   mass presented the higher correlation in Component 2, and was higher in *A. uruguayana*

456   (Table 6) (Fig 5).

457

458   Fig 5. Principal component analysis with log10-transformed of nutrient excretion rates (N and

459   P) and ratio (N:P), body elemental contents (body N, P, C and body N: P, C:N, C:P) and body

460   mass. Arrangement by taxonomy identity of each species. *Macrobrachium borellii* (black

461   circle), *Aegla uruguayana* (dark gray square), *Trichodactylus borellianus* (light gray triangle).

462

463   Table 6. Correlation values excretion rates (N, P) and ratio (N:P), body nutrient contents (N,

464   P, C) and ratios (N:P, C:N, C:P), and body mass on the first and second principal components

465   of PCA-Principal component analysis.

Variable	Correlations of PCA	
	Component 1	Component 2
N excretion	0.4632	-0.4048

23

P excretion	-0.2706	-0.3407
N:P excretion	-0.4847	0.3719
Body N content	0.9264	-0.2331
Body P content	-0.262	-0.749
Body C content	0.7498	-0.02835
Body N:P	0.9116	0.2971
Body C:N	-0.8368	0.3061
Body C:P	0.6150	0.6495
Body mass	-0.1286	0.7587

466

467

Discussion

469 In this study, we assessed which variables best explained nutrient recycling in two scales of

470 analyses (across and within taxa) through empirical data obtained from three freshwater

471 decapod species of different families. Firstly, we analysed the predictive power of physiological

472 (body mass, body elemental content), ecological (diet), taxonomic and experimental (time of

473 incubation) variables on nutrient (N, P, N:P) excretion. Secondly, we focused the analysis on

474 physiological variables to test the hypothesis of ecological theories in our data. We also

475 evaluated and compared the body elemental (C, N, P) content and associated ratios across

476 and within taxa, and used physiological variables as covariates among taxa.

477 **Q1-** Our results showed that the predictive power of variables analysed together

478 changed with the scale of analyses, but the importance of body mass and taxonomic identity

479 on nutrient excretion rates were observed across and within taxa **(H1)**. The time of incubation

480 was also a factor that should be considered in experimental design and, in our case, 30

481 minutes was the most recommended one.

482 **Q2-** When physiological variables (body mass, body elemental content) were analysed

483 independently, body size was a better predictor of nutrient mineralization than body elemental

484   content in both scales of analyses. N:P excretion scaled negatively with body N:P content only

485   across taxa **(H2)**. Allometry was observed across and within taxa, mainly for N excretion; that

486   is, juveniles mineralized more nutrients than adults **(H3)**. Among taxa, the taxonomic identity

487   and body mass had a strong influence in the nutrient N and P excretion while taxonomic

488   identity and body N content had a significant effect on N excretion **(H4)**.

489   **Q3-** Regarding the body elemental content of crustaceans, only body P content of

490   decapods scaled negatively with body mass across taxa **(H5)**, while within taxa, the body N of

491   crabs (negative slope) and body C:N of prawns and crabs (positive slope) exhibited allometry.

492   Among taxa, all elements and ratios were different and a significant effect of body mass was

493   observed on body N, P and C:N. Body C:N was higher in carcinized decapods in comparison

494   with prawns, while body N:P was different in all species and higher in prawns, species with

495   more carnivorous trophic habit **(H6)**. We discussed the implications of the main results, their

496   relations with ecological theories and the usefulness in productive aquatic systems such as

497   IMTA.

498   **Q1** - Several studies have shown that body size (by extension, body mass) is the best

499   predictor of N and P excretion rates in fish, decapod crustaceans and other invertebrates

500   [18,20,22,38,100,101]. This relationship is even more marked in the excretion of N than in P

501   [6], such as observed in the present study. The results obtained by [20] and [6] highlighted the

502   primary importance of MTE in predicting nutrient flux through organisms over other predictors,

503   such as the body elemental composition and diet. Physiological variables like body mass are

504   unfailingly related to taxonomic identity, as corroborated in the present study. For example,

505   the rate at which nutrients are assigned to processes such as reproduction, growth and

506   feeding events are conserved and characteristic at a certain taxonomic level [3,20]. In this

507   way, it is expected that differences observed within species must be due to specific features

508   of life cycle and trophic habits [47,54] of prawn, anomuran and crab species studied here.

509   We also noticed changes in excretion rates over time in both scales of analyses. The

510   nutrient excretion rate was higher during the first 30 minutes of incubation and decreased at

511   60 minutes. This difference was significant for N excretion across taxa and in *A. uruguayana*

25

512   within taxa. Previous studies also reported reduction of nutrient excretion rates as regards

513   time and, in general, authors attributed the reduction in mineralization rates with time due to

514   fasting and handling stress [15,21,22,92]. In our case, crustaceans were acclimated to

515   experimental conditions during 2 weeks, and fed the same diet during this period and just

516   before the excretion trial. In this way, we could infer that differences found in N excretion time

517   had low bias due to fasting and handling stress, and that 30 minutes was an adequate

518   incubation period to make comparative analyses with the studied crustaceans. [21] and [15]

519   also recommended this time lapse as the most appropriate minimum period for the excretion

520   rates to stabilize. The significantly predictive power of time on N excretion rate, which was not

521   observed in P excretion, could be due to the animals' necessity to rapidly remove toxic

522   ammonia from protein metabolism, which negatively influences the organisms' homeostasis

523   [102].

524   The other evaluated variables –diet and body composition– had no effect on the

525   variations in nutrient excretion rates across taxa. Within taxa, only body N content of *M. borelli*

526   positively affected N excretion. Regarding diets used in excretion trials, they had formulations

527   for omnivorous and detritivorous fish that did not differ in N and P content, allowing the two

528   groups of data to be analysed together. Additionally, crustaceans fed the same diet for 2

529   weeks, so it was expected that excretion rates were not influenced by the variable trophic

530   resource, as it commonly occurs in field experiments [103]. Therefore, it was expected that

531   changes in nutrient excretion were related to body elemental composition, which was not true

532   either.

533   The importance of diet and body elemental composition in predicting excretion rates

534   are key concepts in EST, but our results showed that these variables have little or no predictive

535   power to our data. [15] and [20] also found that diet and body elemental content were not good

536   predictors of excretion rates. [17] concluded that diet was not a good predictor, and both N

537   and P excretion rates did not reflect the trophic position (determined by δ^{15}N signatures) of

538   shrimp and fish species. In the case of *M. borellii*, within taxa analyses showed that the body

539   N content of this prawn positively affected N excretion rate, contrary to the EST predictions.

540   However, this regression was not linear and results should be corroborated with excretion

541   trials that evaluate nutrient excretion rates against the consumption of diets with variable N

542   and P composition.

543   **Q2** - The independent analyses of physiological variables supported body mass as a

544   better predictor of nutrient mineralization than body elemental content, both across and within

545   taxa, emphasizing the results found for question 1 (Q1). As previously predicted by MTE, the

546   relationship between these variables is negative [3,7,10] and exhibited variable slopes and

547   explanation percentage at the temperature (24 ± 1 °C) used in the experiments. While body

548   mass explained 45% and 13% of variation in the excretion rates of N and P across taxa,

549   respectively, body N content only explained 36% of N excretion and exhibited a positive slope,

550   which contradicts the EST predictions. On the contrary, body N:P content explained 18% of

551   N:P excretion with a negative slope, fulfilling what was predicted by the EST, despite the low

552   explanation. When we explored results within taxa, all species presented a negative and

553   significant relation between N excretion and body mass, and the regression for *A. uruguayana*

554   presented higher explanation (85%) than the other crustaceans. In this species, body mass

555   also explained 65% of the P excretion rates.

556   The greater predictive power of the body mass in comparison to body elemental

557   content is in concordance with previous studies [6,20] that found little support for the

558   predictions of EST in aquatic organisms. This is reasonable since the excretion of waste

559   nutrients is related to the acquisition and assimilation of food, both processes driven by

560   metabolic demands that change during the life cycle of the organisms [6,7]. Although several

561   studies highlighted the effect of body elemental content on excretion rates in various groups

562   of animals [13,38], for the analysed crustacean species, this trait has a weak predictive value

563   [6,18,20].

564   Although MTE predictors (body mass) explained more variance than EST predictors

565   (body elemental content), the scaling coefficients (regression slopes) revealed variable values

566   in both scales of analysis. Across taxa, slopes were -0.68 for N and -0.49 for P, showing a

567   steeper decrease of N excretion rates than P as crustaceans grew. Within taxa, significant

568 values ranged from -0.56 to -0.75 for N, and -0.67 for P in *A. uruguayana*. In this case, only

569 anomurans exhibited significant relation between both N and P excretion and body mass with

570 higher slope values than the other species. These results indicated that juveniles of the three

571 crustaceans species mineralized more N than adults, and that only juveniles of *A. uruguayana*

572 mineralized more P than adults. The excretion rate per unit mass of juveniles, which is higher

573 than adults (negative slope), was also observed in other crustaceans, mainly for N [18,22,38].

574 The steeper or shallower scaling of excretion rates observed in the three crustacean species,

575 and under the consumption of the same diet, is complex to explain only from an MTE

576 perspective [6]. Body mass and taxonomic identity are not independent, and determining the

577 relative contribution of each one in the variation of excretion rates is a challenge [10].

578 The analyses performed among taxa emphasized that the taxonomic identity had a

579 strong influence in the nutrient excretion rates using both body mass and body elemental

580 content as covariates. These last variables influenced N excretion, while in body elemental

581 content, they only influenced P excretion. In prawns, body N content and body mass

582 influenced the N excretion, differing from crabs and anomurans. Contrary to what would be

583 expected from the EST predictions, prawns mineralized higher N than the other species, even

584 with higher body N content and after the consumption of diets that did not fulfill the protein

585 requirement of this species to grow (~35%) [60]. *A. uruguayana* anomuran and *T. borellianus*

586 crab released the intermediate and the lowest rates of N, respectively, showing the same trend

587 in their respective amounts of body N; results that do not either align with EST predictions [8].

588 These results are surprising and, as suggested by [104] in armour fish taxa, could be related

589 to the variation in the assimilation efficiency, and even in variations in the phenotype

590 expression given the high individual variability observed mainly in the N excretion of prawns.

591 Future studies should include egestion rates of these organisms and feed digestibility

592 to help understand differences in assimilation efficiency in each taxon [105]. These feeding

593 trials would also be helpful to quantify elemental imbalances between body and diet, and to

594 verify the homeostasis regulation of these species [8]. Such trials should consider the

595 residence time and turnover of elements in crustaceans' tissues [106] in the experimental

28

596   design. Regarding P excretion rates, *T. borellianus* excreted higher amounts than *M. borellii*

597   (lower values) and *A. uruguayana.* In heterotrophic organisms, the assimilated P is stored as

598   polyphosphate rich in energy [107,108]. Therefore, prawns and anomurans would assimilate

599   a greater amount of this nutrient because they are more active than crabs and might have

600   higher energy expenditure in mobility.

601   Despite the complexity to understand the differences observed in excretion rates

602   among species and the need to complement the results with additional studies, our findings

603   emphasize the importance of the taxonomic identity and the resolution to analyse animal-

604   mediated nutrient cycling [10]. The digestive morphology and physiology are useful tools that

605   play an important role in the excretion process and that could aid understanding the variations

606   in the studied species and during ontogeny. Juveniles of *T. borellianus* and *A. uruguayana*

607   ingest more animal items than adults [55,58,109], while *M. borellii* seem to maintain a

608   preference to animal components during their entire life cycle [54]. Morphological traits of

609   chelae and mandibles of *A. uruguayana* are different between juveniles and adults [109] and,

610   in *T. borellianus,* feeding apparatus are adapted to cope with dietary changes [63,110]. As

611   regards digestive physiology, juveniles of *A. uruguayana* exhibited higher amylase activity

612   than adults [68] and the metabolic profile varied with the season and ontogeny [67]. In *M.*

613   *borellii*, the proteinase activity is smaller in juveniles than in adults [68].

614   The mentioned background information, together with the results of the present study,

615   provides a deeper insight into feeding strategies, nutritional requirements and resource

616   utilization. This allows a thorough comprehension of the role of the studied crustaceans in the

617   cycling of nutrients in natural aquatic systems, in order to estimate the bioremediation

618   efficiency of decapods in IMTA systems, in later studies.

619   **Q3 -** Across taxa analysis showed that crustaceans exhibited a negative relationship

620   between the body P content and body mass with low explanation (18%). The fact that, at order

621   scale, crustaceans seem to follow the GRH hypothesis –which proposes that small-bodied

622   organisms tend to have faster growth rates and higher body P allocation to protein synthesis

623   [8,11] than larger organisms–, the effect was not observed within taxa. This might have

29

624   occurred because, when all species are analysed together, mass range is wider and sufficient

625   to observe a difference in body P allocation between organisms of lower and higher mass;

626   that is, low body mass species have higher P content and vice versa. This explanation also

627   makes sense if we consider that the slope of regressions within taxa is close to zero; that is,

628   if there is no difference in the body P content between juveniles and adults within taxa, the

629   relationship between body mass and body P content across taxa is an effect of the scale of

630   analyses and must be carefully interpreted. Body mass also poorly explains animal body

631   elemental content in other studies [23,25]. The relationship among these variables is not

632   simple and results found in literature are quite variable in across or within taxa analysis, and

633   even more if data is controlled for phylogenetic effects [23,24,26,111].

634   In our study, within taxa analysis revealed that body N content scaled with body mass

635   in *T. borellianus*. Adult crabs had less N than juveniles and this finding could be related to the

636   relative importance of animal items in the natural diet of this species. Larger individuals

637   consume more vegetal remains than smaller ones, which consume more insect larvae and

638   oligochaetes [56,90]. This feeding habit is also in congruence with the positive allometry of

639   body C:N observed in this crab, since adults eat more C-rich items. We also observed a higher

640   body C:N in adults of *M. borellii*. However, prawns seem to maintain a preference for animal

641   components during the entire life cycle, contradicting this allometric tendency of body C:N

642   content. If there is no evidence that diet could drive variation in body stoichiometry during

643   ontogeny, this relationship could be related to differences in the proportion of chitin between

644   juveniles and adults. Since the chitin of exoskeleton has high C:nutrient [8,27,112-114], adults

645   of both crabs and prawns should have higher amounts of C than N due to more robust

646   carapace. It is necessary to complement these results with studies that explore the influence

647   of diet on body stoichiometry through ontogeny and the deviation from strict homeostasis to

648   get a better understanding of nutrient allocation strategies. We also recommend a

649   complementary study with a broad range of body mass of each species (mainly for *T.*

650   *borellianus*) to confirm the allometric trends of body elemental content found in our study.

651   Regarding among taxa analysis, crustacean species exhibited different body

652   stoichiometry of C, N, P and associated ratios. Prawns had higher body N and C contents,

653   and also lower C:N than anomurans and crabs. These findings could be related to the natural

654   diet and the carnization of crustaceans, since *M. borellii* have a more carnivorous trophic habit

655   [54,57]. This species requires high protein content to achieve better growth parameters [60]

656   and it is an uncarcinized decapod. These traits could contribute to a lower body C:N content

657   compared to the other species, due to a higher ingestion of N-rich food items (higher trophic

658   status) and to a less chitinous carapace. Moreover, digestive enzyme studies revealed that *M.*

659   *borellii* had higher proteinase activity while juveniles of *A. uruguayana* had higher amylase

660   activity in hepatopancreas [68], indicating that metabolism of nitrogen and carbohydrates,

661   respectively, predominates in these organisms. The not intuitive high body C content of *M.*

662   *borellii* could be due to the fact that crustaceans store more lipids in muscle tissue than

663   anomurans [68] and that they also have comparatively more muscle tissue (therefore more

664   body N), which might explain lower values of body C:N. On the other hand, crabs-like body

665   forms species (robust carapace with lower N-rich bodies), such as *T. borellianus* and *A.*

666   *uruguayana*, also consume more algae and vegetal remains than prawns [54,58,106]. The

667   lower trophic status of crabs and anomurans, therefore, exhibited more C:N as expected.

668   Further, body P and N:P content were different among the three species, with *T.*

669   *borellianus* presenting the higher and lower values, respectively. This crab seems to have

670   higher specific growth rates than prawns and anomurans when fed the same omnivorous feed

671   used in this study (Calvo et al., unpublished data). Therefore, higher body P content should

672   be related with a greater allocation of this element to the production of P-rich rRNA to hold

673   faster growth [4] and the lowest N:P ratio is explained by the high P content with respect to N

674   in this species' body. However, this hypothesis should be corroborated by carrying out growth

675   experiments that ensure there is no deficiency in any element regarding the feed formulation,

676   and that it matches the nutritional requirements of tested species.

677

Comments about application in IMTA

678

679 Aquaculture represents almost 50% of the fishery products produced globally and continues

680 to grow faster than other food production sectors, mainly in freshwater systems [115]. The

681 nutrient discharge of this food production system has generated many environmental impacts

682 on ecosystems [116] that could be mitigated and recycled through the diversification and

683 incorporation of complementary species, such as proposed by IMTA concepts [117,118]. In

684 low taxa diversity aquatic systems, like an IMTA, the nutrient turnover is strongly influenced

685 by the few taxa that compose it [81,14]. Therefore, understanding the physiological processes

686 of organisms at different levels of organizations is essential to explore and predict nutrient

687 recycling behavior in different scenarios (unique species and groups of species) and to design

688 integrated productive systems that efficiently use the nutrient inputs through an adjusted mass

689 balance.

690 Decapods could display different roles as organic nutrient recyclers in integration with

691 detritivorous and omnivorous fish. The differences found in N and P excretion rates across

692 and within taxa highlighted that if they were integrated as a unique species or as a group of

693 species, the effect on nutrient recycling could be different. In both cases, we expected that

694 juveniles recycle more nutrients per mass unity than adults, mainly for N. Therefore, early

695 stage decapods would contribute more to transforming N from proteins to ammonium (i.e.

696 reducing organic burden) and enhance the availability of this compound to nitrifying bacteria

697 to finally grow vegetables or algae. Inorganic N could be even more available with the

698 integration of prawns only. On the other hand, prawns incorporate more P and, therefore,

699 could limit this nutrient to primary producers while representing a P-rich by-product for many

700 purposes. Contrary, crabs mineralize less N and more P exerting the opposite effect. These

701 results showed that the role of crustaceans as animal-mediate nutrient recyclers depends

702 mainly on body mass and taxonomic identity and should be considered to select

703 complementary species that efficiently use feed resources.

32

704   Body elemental content is another variable that could be a useful baseline to estimate

705   the nutritional requirements of animals [119,120]. Our study revealed that this variable differed

706   with body mass (ontogeny) and that it is related to the natural trophic status of organisms.

707   Results showed higher N-requirements and higher trophic status (lower C:N) of prawns than

708   anomurans and crabs. Moreover, prawns and crabs change the trophic status during

709   ontogeny, requiring lower N in comparison with C at later stages. Prawns need more protein

710   in the feed and might be successfully integrated with fish of higher N-requirements, while crabs

711   and anomurans might exhibit good performance with fish that are fed lower N-rich diets, such

712   as herbivorous and omnivorous fish.

713   Our study was an effort to contribute to the ecological background of MTE and EST

714   through empirical data obtained from freshwater decapod species with aquacultural potential

715   use, and to provide useful information about nutrient mineralization and nutritional

716   requirements based on body elemental content of these crustaceans. These results, added to

717   other empirical data on egestion, digestibility, retention in biomass (growth and reproduction),

718   and food intake, offer a framework to leave some open questions for further studies and

719   provides information to infer the amount of crustaceans needed to biomitigate the feed

720   remains of detritivorous and omnivorous fish culture, through an improved mass balance.

721   Ecological theories and experimental aquaculture research can be good allies when it is

722   necessary to "imitate" nature and achieve more efficient production processes with less impact

723   on the ecosystem.

724

Acknowledgments

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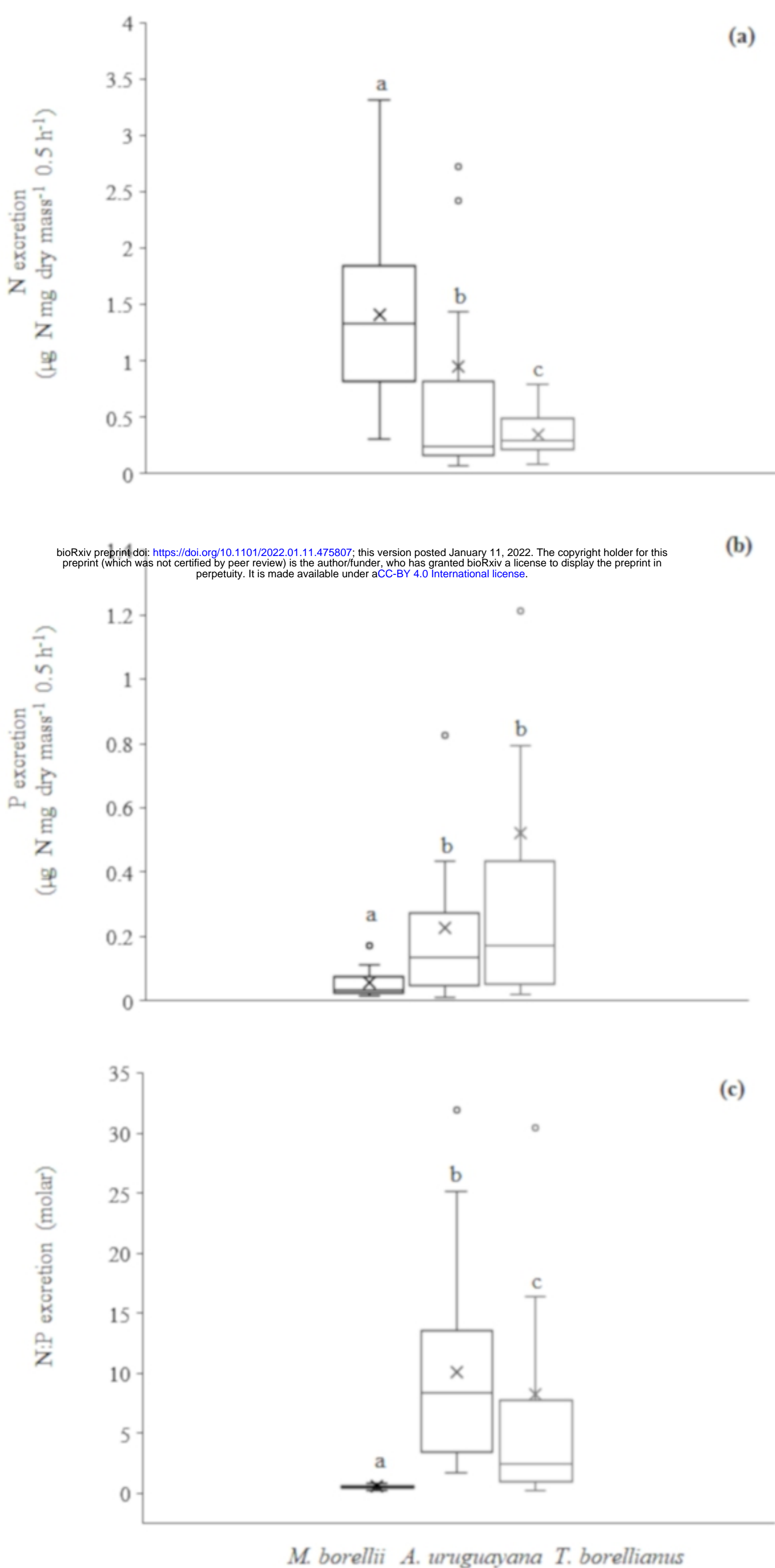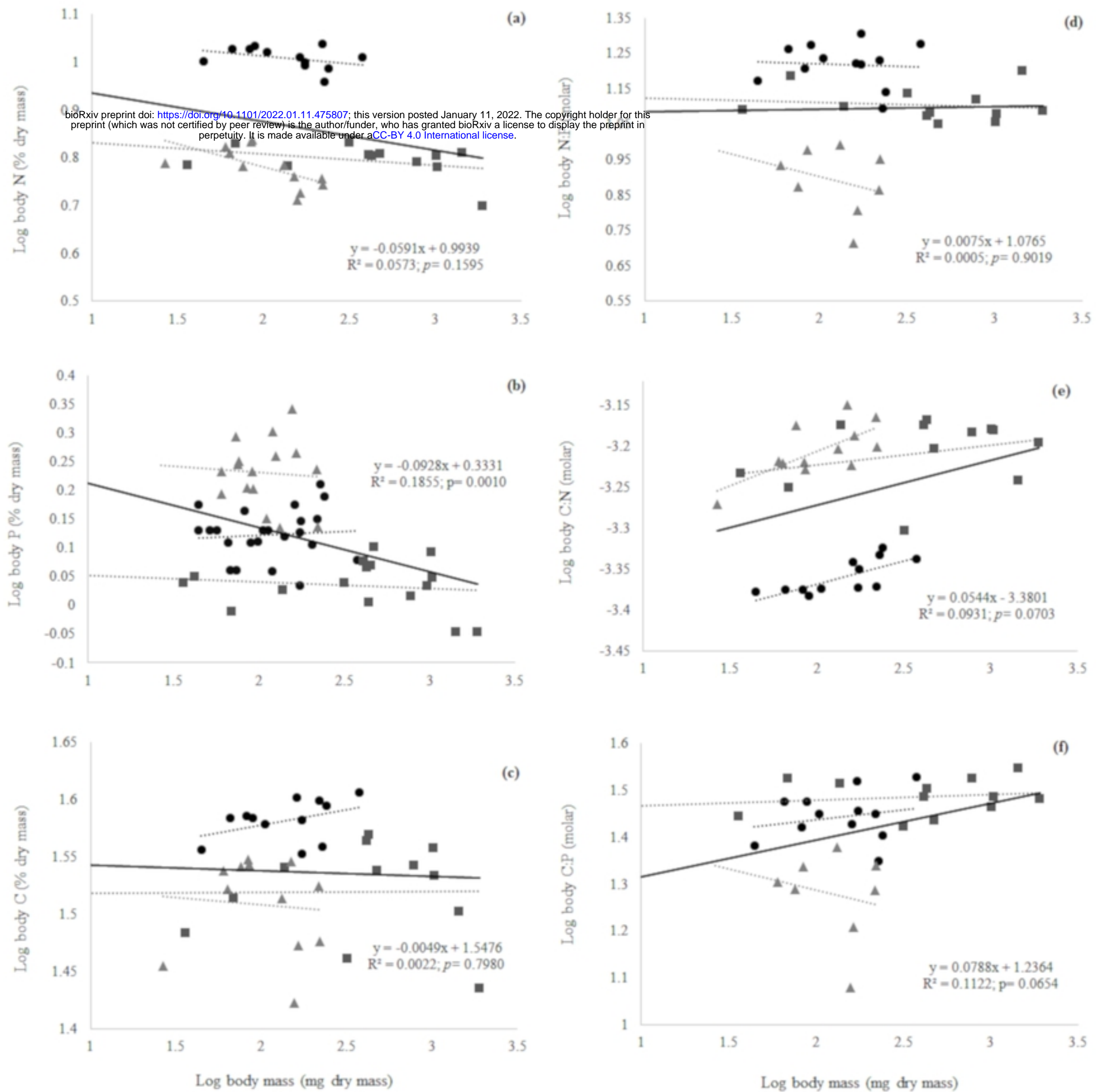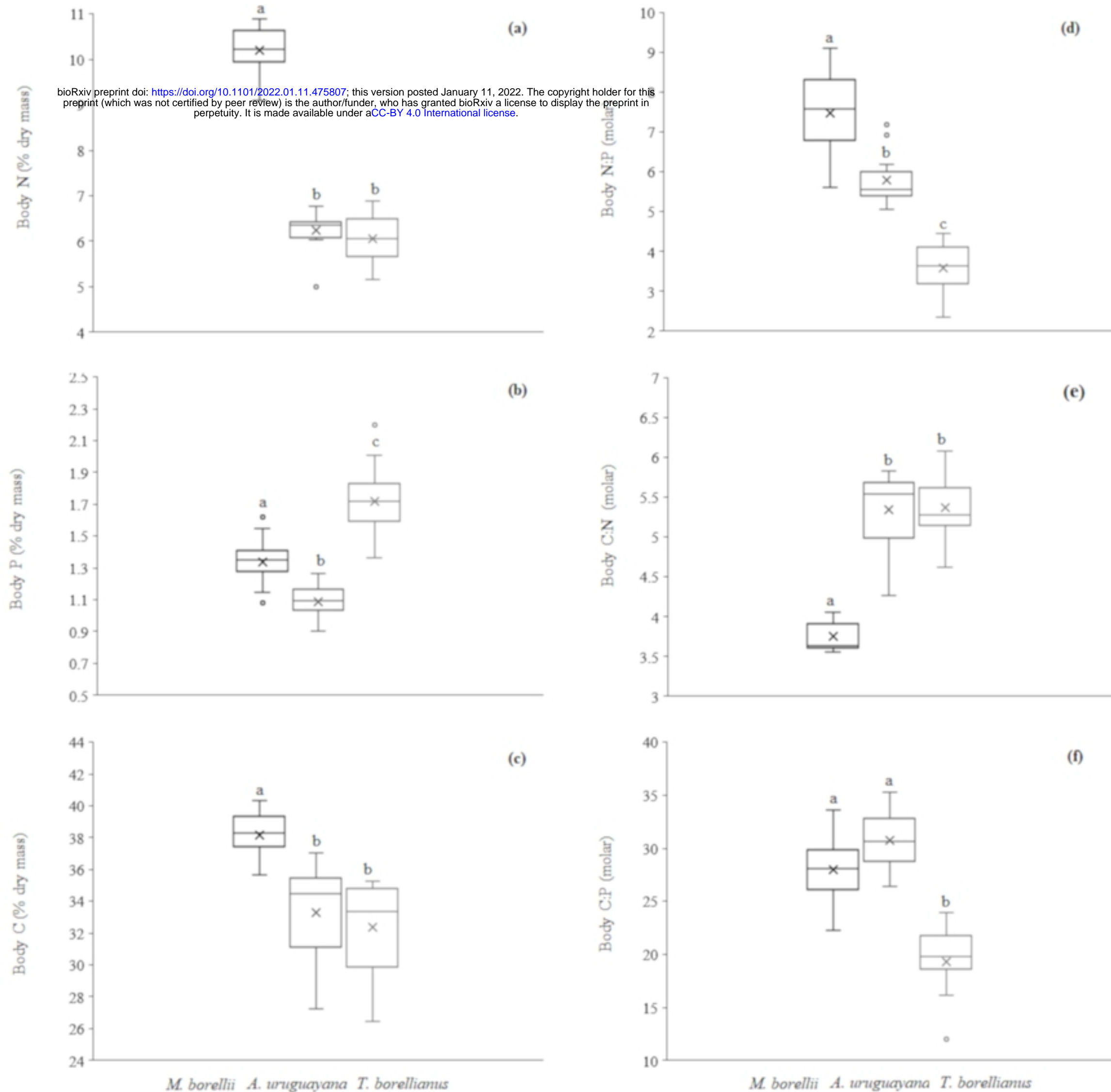Figure 1

(a)

(b)

(c)

M. borellii   A. uruguayana   T. borellianus
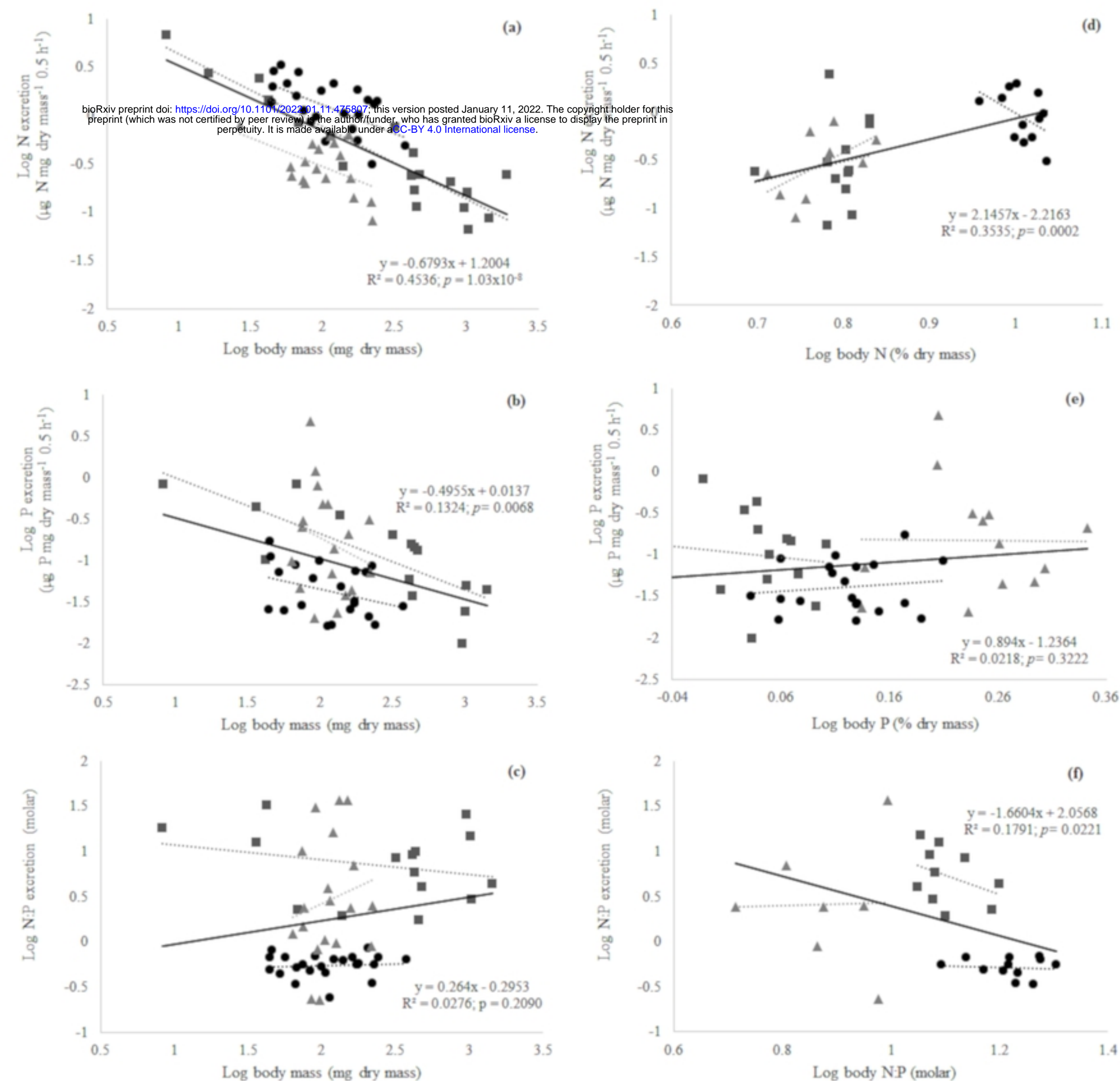
Figure 2

Figure 3

Figure 4

Figure 5