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Mixotrophy upgrades food quality for marine calanoid copepods

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Abstract

Inorganic nutrient limitation affects the stoichiometry and nutritional quality of marine phytoplankton. Mixoplankton, able to photosynthesize and feed simultaneously in the one cell, can compensate shortage of nutrients by phagotrophy, theoretically upgrading their nutritional quality for their predators: the zooplankton. Yet, the additional value that phagotrophy in mixoplankton may provide to support zooplankton growth and recruitment has been poorly explored. Therefore, we investigated the feeding and reproductive performances of the copepods *Paracartia grani* and *Centropages typicus* on mono-diets of the dinoflagellate *Karlodinium veneficum* grown as strict autotroph and as mixotroph, both under N and P depletion, and in nutrient-balanced conditions (f/2 medium; only as autotroph). Feeding and reproduction outputs were generally higher in *P. grani* than in *C. typicus*. Both copepod species ingested the mixotrophic *K. veneficum* at similar rates than the autotrophic ones in either nutrient-limited scenario. However, egg production and recruitment rates generally increased when feeding on mixotrophs, in *P. grani* on both N- and P-limited diets, and in *C. typicus* under P limitation. In general, P limitation influenced copepod physiology more than N depletion. Our results show that phagotrophy upgrades nutritional quality in nutrient-limited mixotrophs as prey for copepods compared to the strict autotrophic ones. These findings are among the first reported cases of copepod ingestion in the laboratory on actively feeding mixoplankton, and they highlight the importance of considering the trophic mode of the protist and the response by various zooplanktonic predators when attempting to understand the functioning of marine planktonic food webs.

Marine ecosystems are experiencing selective inputs of nitrogen (N) and/or phosphorus (P), leading to the establishment of unfavorable seawater stoichiometry (Peñuelas et al. 2013; Romero et al. 2013). Therefore, as seawater and plankton elemental composition influence one another, a difference in seawater N : P ratio is likely to affect the physiology and ecology of photosynthetic plankton with cascading effects on other components within marine food webs (Ryther and Dunstan 1971; Glibert et al. 2005; Romero et al. 2013). Unfavorable seawater stoichiometry can affect microalgal growth, although this condition might be of lesser importance to several phototrophic plankton, such as mixoplankton, as they may acquire nutrients in the organic form through phagotrophy, beside photosynthesis (Stoecker et al. 1987; Hansen et al. 2019). Mixoplankton, including mostly flagellates, dinoflagellates, and ciliates are rather common organisms in marine and freshwater environments (Flynn et al. 2019).

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Copepods, the major link between protist plankton and fish, are known to acquire a great fraction of their metabolic requirements from a dinoflagellate diet, especially during non-diatom bloom periods in temperate and cold seas or all the year round in oligotrophic ecosystems (Kleppel 1993; Saiz and Calbet 2011). Generally, dinoflagellates are of a good nutritional value for copepods as they are source of important biochemical components (proteins, lipids) (Kleppel 1993; Broglio et al. 2003, 2004; Jeong et al. 2010). Yet, when nutrient-stressed, dinoflagellates may result of less nutritional value for copepods as demonstrated in previous studies (Jónasdóttir 1994; Jones and Flynn 2005; Isari et al. 2013). We would expect, however, that a mixotrophic life strategy in dinoflagellates would buffer the inorganic nutrient shortage, ultimately improving their nutritional value for copepods. Nonetheless, there is scarce empirical evidence that corroborates this hypothesis because most of the related studies conducted so far on zooplankton are merely numerical model simulations (Ward and Follows 2016) or experimentation on other zooplanktonic groups, such as freshwater daphniids or rotifers (Katechakis et al. 2005; Weithoff and Wacker 2007; Vad et al. 2020). The few experimental work conducted with copepods and mixoplankton did not address this nutrient buffering effect, and more generally reported very contrasting results regarding the ecological role of mixotrophy in

food-web dynamics. Thus, some authors have observed enhanced copepod growth when the diet was supplied with mixotrophic flagellates (Ptacnik et al. 2004), whereas feeding reduction and reproductive impairment were documented in other studies, despite in most cases the species of interest were grown as mere autotrophs (Jeong et al. 2010; Turner 2014).

In this study, we postulate that nutrient-limited dinoflagellates reared under mixotrophic metabolism could buffer the nutritional shortage imposed by nutrient-deficient medium in contrast to strict autotrophic dinoflagellates that do not engage in phagotrophy. Therefore, contrarily to obligate autotrophy, the mixotrophic strategy in nutrient-limited environment would have the potential to increase dinoflagellate nutritional quality for predators (food upgrade) and therefore benefit the predator's physiological performance. To test this hypothesis, we provided adult females of the calanoid copepods *Paracartia grani* and *Centropages typicus* with mono-diets of *Karlodinium veneficum* grown as pure autotroph or as mixotroph, under nutrient- (N and P) rich and deficient conditions. The widespread constitutive mixoplankter *K. veneficum* has proved to represent a good model organism to test our food upgrade hypothesis due to its ability to grow under both metabolic strategies (photoautotrophy and mixotrophy) as well as it has been previously documented to acquire the insufficient amount of inorganic N and/or P through cryptophyte feeding (Lin et al. 2017). However, to our knowledge, the effects of such trophic plasticity have never been tested on higher trophic levels such as copepods. Given the key position of copepods in marine food webs (Williams et al. 1994; Turner 2004; Steinberg and Landry 2017), the high abundance of mixoplankton in natural systems (Stoecker et al. 2009, 2017), and the expected future global change-related forcings on marine ecosystems, including nutrient imbalances (Peñuelas et al. 2013; Romero et al. 2013), the results of this study will be very relevant to properly parameterize ecosystem predictive models.

Materials

K. veneficum culturing and adaptation to phagotrophy

The *K. veneficum* strain (ICM-K21; Institut de Ciències del Mar, CSIC collection) used in these experiments was isolated from Alfacs Bay (NW Mediterranean) (Calbet et al. 2011), and it has been kept in monoclonal nonaxenic culture in f/2 medium (Guillard 1975) since June 2007, at 38 salinity, 19°C ± 1°C, 10 : 14 light : dark cycle, 50 μmol photons m^{-2} s^{-1}. This original strain of *K. veneficum* was subsequently split in two independent subcultures, one that was maintained in unchanged photoautotrophic conditions (f/2 medium), and another one that was subjected to a 2-year adaptation to phagotrophy, being reared in plain filtered seawater (FSW) with the supply of the cryptophyte *Rhodomonas salina* as prey. *R. salina* was grown in semi-batch cultures kept in f/2 medium in the same temperature and photoperiod conditions as the dinoflagellate but under higher light intensity (100 μmol photons m^{-2} s^{-1}) and harvested for feeding *K. veneficum* during the exponential phase.

Copepod stock cultures

P. grani and *C. typicus* originate from specimens collected in waters near Barcelona (Saiz et al. 2015; Olivares et al. 2020) and maintained in culture (19°C ± 1°C, 38 salinity) at the Institut de Ciències del Mar since 2007 and 2018, respectively. The experimental cohorts were established by siphoning out the bottom of mature cohorts and transferring the newly laid eggs into 18 liters tanks filled with 0.1 μm FSW with constant gentle aeration. The *P. grani* and *C. typicus* cultures were maintained with a supply of, respectively, the cryptophyte *R. salina* and the heterotrophic dinoflagellate *Oxyrrhis marina*; in the case of *C. typicus*, the autotrophic dinoflagellate *Heterocapsa* sp. was supplied in addition to *O. marina* to support naupliar growth. The amount of prey offered was adjusted along ontogenetic development to ensure non-limiting concentrations based on previous knowledge (Olivares et al. 2020). Both *R. salina* and *Heterocapsa* sp. were grown in semi-continuous batch cultures in f/2 medium and supplied with constant aeration, whereas *O. marina* was reared in FSW on a daily diet of *R. salina*. All prey used for maintenance of copepod cohorts were harvested during exponential growth.

Experimental design and procedures

We studied the physiological response of *P. grani* and *C. typicus* adult females to *K. veneficum* grown under autotrophic and mixotrophic conditions and different inorganic nutrient loads (limited vs. replete). Overall, each experiment consisted of three major phases: conditioning of the dinoflagellate to nutrient concentrations (duration: 6 d), followed by the copepod acclimation to prey type and nutritional condition (4 d), then a 24-h experimental incubation to determine copepod ingestion and egg production, and finally an extra 48 h to assess egg hatching success (Fig. 1). Due to the high number of samples to manipulate simultaneously, experiments were carried out in different days with recently molted adults (similar age) from different cohorts.

During the dinoflagellate conditioning phase, we grew *K. veneficum* under five different treatments: two of them were mixotrophic *K. veneficum* cultures reared in N and P limitation by a dilution factor of 10 (N/20 and P/20), but keeping the non-limiting nutrient in f/2 amounts. The remaining three treatments were all autotrophic *K. veneficum* and included the same nutrient-limited treatments (N/20 and P/20) and additionally a nutrient-repletion one (f/2 medium). In the case of mixotrophic *K. veneficum* lines (N/20 and P/20), cultures were also provided with food (*R. salina*), replenished on a daily basis up to a prey : predator ratio of 1 : 1; *R. salina* was previously centrifuged (2291 rpm, 1000 × *g*, 10 min) to avoid introducing external nutrients into the *K. veneficum* cultures. The elemental contents of the *R. salina* (ESD: 7.54 μm) offered to the mixotrophic *K. veneficum* cultures were as follows: pg C

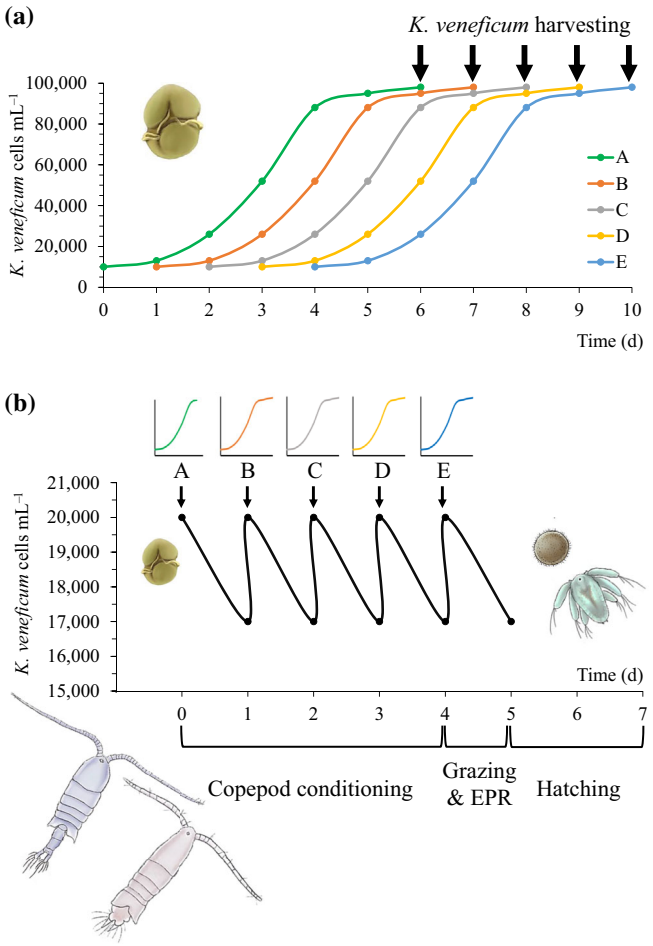

Fig 1. Timeline of experimental phases for (**a**) *K. veneficum* culturing and (**b**) copepod experiments. (**a**) Simplified scheme of the growth of five different cultures of *K. veneficum* started with 1-d delay. Each nutrient and trophic mode treatment has its own series of five cultures, here defined as A, B, C, D, E. After 6-d growth, each *K. veneficum* batch cultures was harvested and used to feed copepods during the phase of conditioning, replenished on a daily basis, and in the grazing and egg production incubation (between days 4 and 5).

μm^{-3}: 0.18 ± 0.002, pg N μm^{-3}: 0.038 ± 0.0002, pg P μm^{-3}: 0.0038 ± 0.00004 (C : N : P = 123 : 22 : 1). The last *R. salina* input to the mixoplanktonic *K. veneficum* cultures was administrated 24–48 h before dinoflagellate harvesting to ensure the absence of cryptophyte cells in *K. veneficum* culture the day of copepod feeding. The concentration of *R. salina* in *K. veneficum* cultures was checked with a Multisizer particle counter (Beckman Coulter); non-significant amounts of *R. salina* were detected in most of occasions, ensuring no contribution of the cryptophyte to the copepod diet. For each of the five nutritional treatments, five *K. veneficum* batches with 1-d difference were inoculated in order to use prey of similar physiological condition throughout the copepod acclimation and incubation phases (Fig. 1a), similarly to the procedure described in Isari et al. 2013. All cultures were kept under the same abiotic conditions of irradiance (100 μmol photons m^{-2} s^{-1}), temperature (19°C ± 1°C) and photoperiod (10 : 14 light : dark cycle) and grown in 1, 2, or 5 liters autoclaved Pyrex bottles according to volume needs. Each of the abovementioned batches were let grow during 1 week (day 0 to day 6), during which cell concentration and cell size were monitored daily with the particle counter. In addition, samples for inorganic nutrient concentration were collected every day of the monitoring period. Harvesting occurred at day 6, when cultures were expected to be inorganic nutrient- and prey-depleted according to previous trials, with the exception of the autotrophic *K. veneficum* reared in f/2 medium, which was harvested when in exponential growth phase.

At the onset of the copepod acclimation phase, recently molted (< 15–18 d) adult individuals (including males and females, 1 : 1 ratio approximately) of *P. grani* and *C. typicus* were subsampled from copepod stock culture tanks by collecting aliquots of known number of organisms. These were gently collected onto 200 μm mesh and promptly transferred into 4-liter Nalgene polycarbonate bottles, previously filled with *K. veneficum* suspensions of the different nutritional treatments (autotrophic diets: f/2, N/20, P/20, and mixotrophic diets: N/20, P20) were prepared on the corresponding harvesting day (day 6 of the dinoflagellate growth curve, early stationary phase). The food concentration used for copepod conditioning and subsequent experimental incubation (Table 1) were replenished on a daily basis and were above satiation levels for both *P. grani* (9932 ± 948 cells mL^{-1}; 884 ± 87 μg C L^{-1}) (Isari et al. 2013) and *C. typicus* (27,738 ± 2187 cells mL^{-1}; 2346 ± 335 μg C L^{-1}) (Tomasini and Mazza 1978). The number of copepods kept in the 4-liter acclimation bottles ranged between 246 and 252 for *P. grani* and between 328 and 385 for *C. typicus*, and was set according to the clearance rate estimated for each copepod species on *K. veneficum*. After the 4 d of copepod acclimation, both copepod species were tested for ingestion, egg production and egg hatching rates (Fig. 1b). The *K. veneficum* conditioned stocks were checked for concentration with the particle counter and 10-liter fresh food suspensions at the desired prey concentrations were prepared (Table 1). Each food suspension was gently mixed and distributed homogeneously in nine 613 mL Pyrex bottles, including one start bottle, four control (without copepods), and four experimental bottles (with copepods). Once the bottles had been filled, female individuals were sorted from the conditioning Nalgene bottles and groups of 15 (*P. grani*) or 20 (*C. typicus*) adult females were transferred with a wide-mouth glass pipette into each of the four experimental Pyrex bottles. Only healthy and actively moving females were selected for incubations. Bottles were filled up to the top, capped with Teflon-lined screw-caps, and placed end-over-end on a rotating plankton wheel at 0.2 rpm. Start bottles were sampled just after filling, in order to determine the initial prey concentration and cell size with the particle counter of the different dietary treatments.

After 24 h incubation, control and experimental bottles were removed from the rotating wheel, gently mixed and the

Table 1. Prey concentrations, both in cells and carbon terms, in the copepod incubations. Mixo, mixotrophic; Auto, autotrophic; N/20, N-limited; P/20, P-limited; f/2, nutrient-balanced. Data are average ± SE of the mean.

Copepod	Treatment	Prey concentration (cells mL^{-1})	Prey concentration (μg C L^{-1})
P. grani	Auto f/2	10,720±42.1	693±2.7
P. grani	Auto N/20	12,554±39.1	733±2.3
P. grani	Auto P/20	10,887±35.5	829±2.7
P. grani	Mixo N/20	7894±46.2	1012±5.9
P. grani	Mixo P/20	7607±56.6	1154±8.6
C. typicus	Auto f/2	30,485±166.0	1743±9.5
C. typicus	Auto N/20	33,081±31.4	1935±1.8
C. typicus	Auto P/20	30,058±104.7	1776±6.2
C. typicus	Mixo N/20	22,541±46.8	2878±6.0
C. typicus	Mixo P/20	22,525±70.5	3397±10.6

content was sieved through 200 and 37 μm meshes to collect copepods and their eggs, respectively. The control bottles (without copepods) were treated similarly for methodological consistency. The filtrate containing only *K. veneficum* cells was then gently homogenized and sampled to determine cell concentration and size with the particle counter. The copepods from the incubation bottles were collected in Petri dishes, then checked for mortality or signs of impairment, and finally anesthetized with MS-222 to be counted and processed for elemental analysis (see next subsection). Eggs samples were transferred to Petri dishes and let incubate for 48 h at 19°C to assess hatching success. After 48 h, the samples were preserved with 2% acetic Lugol's solution, and eggs and nauplii counted under the stereomicroscope to determine copepod egg production rates and egg hatching success.

Elemental content determinations

During both the copepod conditioning and the experimental incubation phases, *K. veneficum* stocks used to prepare the fresh prey suspensions were sampled and filtered onto precombusted Whatman 25 mm GF/C filters (450°C, 5 h) for particulate C, N, and P content analysis. CN filters were ovendried (60°C, 48 h) and stored in a desiccator until analysis (Flash EA1112, Thermo Finnigan), whereas P filters were immediately frozen (−80°C) until processing. Phosphate digestion (orthophosphate acid persulfate oxidation) technique was applied to convert particulate organic P into inorganic dissolved P and then analyzed using a Seal Analytical AA3 (Bran + Luebbe) analyzer. The filtrates obtained after GF/C filtering were also collected to assess the inorganic nutrient concentration in the medium as explained above.

Copepod females that had been left in the Nalgene conditioning bottles and those retrieved from the experimental Pyrex bottles at the end of the incubation were collected, concentrated in Petri dishes and anesthetized with MS-222. They were photographed (40× magnification, LEICA-MC170 HD) to estimate prosome length and then placed onto precombusted Whatman 25 mm GF/C filters for the determination of copepod CNP content and stoichiometric ratios. Between 15 and 20 individual copepods were transferred to each filter (in tri- or quadruplicates) for the determination of CN and P measurements per each dietary treatment. Filters for copepod particulate CNP content were stored and processed as for protists.

Data analysis and statistics

Copepod ingestion rates (cells ind^{-1} d^{-1}) were calculated using Frost's equations (Frost 1972) and converted to C, N, and P intakes using the volumetric prey content determined in this study. Egg production rate (EPR, eggs ind^{-1} d^{-1}) was converted into carbon units assuming egg content of 38 ng C egg^{-1} and 29 ng C egg^{-1} for respectively *P. grani* (Saiz et al. 2020) and *C. typicus* (Saiz unpubl.). Hatching success, estimated after 48 h incubation, was expressed as the percentage of nauplii hatched relative to the total offspring. Daily naupliar recruitment rate (nauplii ind^{-1} d^{-1}) was calculated as the product of egg production rate and hatching success. Finally, the copepod carbon gross-growth efficiency (C-GGE) was expressed as the quotient between egg production and ingestion rates expressed in C units (expressed as a fraction of 1). Data were statistically analyzed and graphically explored with the software R studio (v. 3.6.2), Sigma-Plot v.14.0 and Prism 7. Statistical analysis typically included one-way ANOVA and two-way ANOVA tests followed by Bonferroni tests for the planned pairwise comparisons among either the autotrophic prey (N- and P-limited vs. f/2 treatment) or between autotrophic and mixotrophic limited prey (auto N/20 vs. mixo N/20, and auto P/20 vs. mixo P/20). For clarity, we only present in the text the outcome of the pairwise comparisons and the adjusted probability values. Supplementary Tables S1–S4 show the data of the ANOVA tests carried out. Data were checked for normality and homoscedasticity using Shapiro–Wilk and Brown–Forsythe tests, respectively. In a few cases, data were log transformed to correct for heteroscedasticity. Hatching success data (%) was transformed (arcsine of square root) previous to statistical analysis.

Results

Prey and predators characterization

The cell size, elemental content, and stoichiometric molar ratios of *K. veneficum* used in the experiments reflected both the mixotrophic character and the nutritional limitation of the treatments applied (Table 2). Supplementary information on the batch growth of *K. veneficum* and temporal evolution in the nutrient load of the growth medium can be found in Supporting Information Fig. S1. Mixotrophs were slightly larger than the corresponding nutrient-depleted autotrophs, and had higher elemental content, resulting in significantly differences in the stoichiometric molar ratios of the prey offered in the experiments (Table 2). C : N ratios of autotrophic *K. veneficum* were overall significantly higher under nutrient limitation compared to f/2 treatments, both in the *P. grani* (Table 2; Bonferroni tests: auto f/2 vs. auto N/20: $p < 0.001$, auto f/2 vs. auto P/20: $p < 0.001$) and *C. typicus* experiments (Table 2; Bonferroni tests: auto f/2 vs. auto N/20: $p = 0.084$, auto f/2 vs. auto P/20: $p < 0.001$). Nutrient-limited mixotrophs also showed differences in C : N ratios with their autotrophic counterparts, particularly between the N-limited treatments (Table 2; *P. grani* experiments: Bonferroni tests: auto N/20 vs. mixo N/20: $p < 0.001$, auto P/20 vs. mixo P/20: $p > 0.9$; *C. typicus* experiments: Bonferroni tests: auto N/20 vs. mixo N/20: $p < 0.001$, auto P/20 vs. mixo P/20: $p < 0.001$). C : P molar ratios differed also among treatments (Table 2). Thus, among the autotrophic diets C : P ratios were largely increased as a consequence of P-limitation, although N-limitation also moderately affected *K. veneficum* C : P ratio in comparison with the f/2 treatment (Table 2; *P. grani* experiments: Bonferroni tests: auto f/2 vs. auto N/20: $p = 0.032$, auto f/2 vs. auto P/20: $p < 0.001$; *C. typicus* experiments: Bonferroni tests: auto f/2 vs. auto N/20: $p = 0.085$, auto f/2 vs. auto P/20: $p < 0.001$). Mixotrophic *K. veneficum* showed some differences in their C : P ratios compared to the autotrophic forms when P-limited, but without a consistent trend (Table 2; *P. grani* experiments: Bonferroni tests: auto N/20 vs. mixo N/20: $p = 0.166$, auto P/20 vs. mixo P/20: $p = 0.015$; *C. typicus* experiments: Bonferroni tests: auto N/20 vs. mixo N/20: $p = 0.156$, auto P/20 vs. mixo P/20: $p = 0.002$). Similar to C : P ratios, N : P ratios among the autotrophic diets were much higher in the P-limited treatment, and N-limitation provided less consistent changes (Table 2; *P. grani* experiments: Bonferroni tests: auto f/2 vs. auto N/20: $p = 0.138$, auto f/2 vs. auto P/20: $p < 0.001$; *C. typicus* experiments: Bonferroni tests: auto f/2 vs. auto N/20: $p = 0.047$, auto f/2 vs. auto P/20: $p < 0.001$). The comparison of the autotrophic and mixotrophic nutrient-limited forms showed significant, but moderate differences, with no consistent patterns (Table 2; *P. grani* experiments: Bonferroni tests: auto N/20 vs. mixo N/20: $p = 0.014$, auto P/20 vs. mixo P/20: $p = 0.014$; *C. typicus* experiments: Bonferroni tests: auto N/20 vs. mixo N/20: $p = 0.004$, auto P/20 vs. mixo P/20: $p = 0.141$).

Carbon contents of the *P. grani* used in the experiments were similar among treatments (Table 3; Bonferroni tests: $p > 0.6$ in all planned comparisons, both among autotrophic diets and also between auto vs. mixo nutrient-limited diets). The *C. typicus* adult individuals from the cohort used on the mixotrophic diets were smaller and had lower carbon content than the other specimens used (Table 3; Bonferroni tests: auto f/2 vs. auto N/20: $p = 0.289$, auto f/2 vs. auto P/20: $p = 0.139$; auto N/20 vs. mixo N/20: $p < 0.001$, auto P/20 vs. mixo P/20:

Table 2. Size (ESD), elemental contents, and molar stoichiometric ratios of the *K. veneficum* cultures in the different copepod experiments. Mixo, mixotrophic; Auto, autotrophic; N/20, N-limited; P/20, P-limited; f/2, nutrient-balanced. Brackets indicate predator used (P: *P. grani*; C: *C. typicus*). Notice the mixotrophic *K. veneficum* cultures were common for the experiments with both copepod species. Data are average ± SE of the mean.

Treatment	ESD (μm)	C contents (pg C 10^3 μm^{-3})	N contents (pg N 10^3 μm^{-3})	P contents (pg P 10^3 μm^{-3})	C : N	C : P	N : P	C : N : P
Auto f/2 (P)	9.9±0.023	101±1	24±0.1	9.7±0.49	5.0±0.04	27.0±0.30	5.4±0.03	27:5:1
Auto N/20 (P)	10.2±0.003	106±2	22±0.6	7.5±0.08	5.6±0.07	36.7±0.56	6.5±0.17	37:7:1
Auto P/20 (P)	10.7±0.004	119±3	21±0.5	2.8±0.01	6.6±0.04	109.9±3.19	16.7±0.39	110:17:1
Auto f/2 (C)	10.2±0.011	89±3	22±0.4	9.3±0.01	4.7±0.07	24.6±0.94	5.2±0.10	25:5:1
Auto N/20 (C)	10.5±0.032	98±3	23±0.7	8.1±0.02	4.9±0.01	31.3±0.98	6.4±0.20	31:6:1
Auto P/20 (C)	10.8±0.023	92±6	19±1.2	2.7±0.01	5.7±0.05	88.0±6.08	15.5±0.96	88:15:1
Mixo N/20 (P+C)	11.7±0.019	154±8	29±1.6	12.9±0.07	6.2±0.03	30.1±1.57	4.9±0.27	30:5:1
Mixo P/20 (P+C)	11.7±0.010	181±4	32±0.7	4.9±0.01	6.6±0.03	95.7±2.00	14.6±0.32	96:15:1

Table 3. Copepod size (prosome length) and carbon contents. Mixo, mixotrophic; Auto, autotrophic; N/20, N-limited; P/20, P-limited; f/2, nutrient-balanced. Data are average ± SE of the mean.

Copepod	Treatment	Size (μm)	C contents (μg C ind^{-1})
P. grani	Auto f/2	976±4.5	4.0±0.34
P. grani	Auto N/20	952±6.8	3.8±0.07
P. grani	Auto P/20	967±4.6	3.7±0.26
P. grani	Mixo N/20	932±13.7	3.8±0.15
P. grani	Mixo P/20	923±11.3	4.0±0.20
C. typicus	Auto f/2	962±7.1	5.8±0.16
C. typicus	Auto N/20	952±8.3	5.4±0.14
C. typicus	Auto P/20	943±6.4	5.3±0.22
C. typicus	Mixo N/20	909±12.8	3.9±0.17
C. typicus	Mixo P/20	903±6.3	4.6±0.09

$p = 0.027$). To account for these differences in further comparisons, rates expressed on a per individual basis were normalized to a common average body carbon using an allometric scaling of 0.743 (Saiz and Calbet 2007).

Copepod ingestion rates

P. grani ingested cells of the autotrophic f/2 *K. veneficum* at higher rates than N- and P-depleted autotrophic diets, but differences were only significant when compared with the N/20 treatment (Fig. 2a; Bonferroni tests: auto f/2 vs. auto N/20: $p = 0.018$, auto f/2 vs. auto P/20: $p = 0.15$). When comparing the mixotrophic and autotrophic nutrient-depleted diets, no different cellular intake was registered (Fig. 2a; Bonferroni tests: auto N/20 vs. mixo N/20: $p > 0.9$, auto P/20 vs. mixo P/20: $p = 0.066$). In terms of carbon, ingestion of *P. grani* offered autotrophic food were similar between the f/2 and P- limited diets, and decreased in the N/20 treatment (Fig. 2b; Bonferroni tests: auto f/2 vs. auto N/20: $p = 0.002$, auto f/2 vs. auto P/20: $p > 0.9$). Contrarily, mixotrophs were ingested at much higher rates in both N- and P-limitation treatments than the autotrophic counterparts (Fig. 2b; Bonferroni tests: auto N/20 vs. mixo N/20: $p < 0.001$, auto P/20 vs. mixo P/20: $p = 0.005$). Nitrogen intake of *P. grani* was significantly reduced in the autotrophic N/20 diet, whereas differences were not significant for the autotrophic P/20 diet (Fig. 2c; Bonferroni tests: auto f/2 vs. auto N/20: $p < 0.001$, auto f/2 vs. auto P/20: $p = 0.004$). Mixotrophic diets proved, however, to increase N intake for *P. grani* in both limited diets in comparison with their autotrophic counterparts (Fig. 2c; Bonferroni tests: auto N/20 vs. mixo N/20: $p < 0.001$, auto P/20 vs. mixo P/20: $p = 0.006$). Regarding P intake, both N/20 and P/20 autotrophic *K. veneficum* resulted in severe reduction in *P. grani* ingestion rates compared to the f/2 autotrophic diet (Fig. 2d; Bonferroni tests: auto f/2 vs. auto N/20: $p < 0.001$, auto f/2 vs. auto P/20: $p < 0.001$). Mixotrophic diets resulted in enhancement of P intake in comparison with the corresponding nutrient-limited autotrophic dinoflagellates, although differences were only significant for the N/20 treatment (Fig. 2d; Bonferroni tests on log transformed data: auto N/20 vs. mixo N/20: $p < 0.001$, auto P/20 vs. mixo P/20: $p < 0.001$).

In *C. typicus* the lowest cell ingestion rate was recorded on a diet of the autotrophic f/2 dinoflagellate, but it was not significantly different from both depleted autotrophic diets (Fig. 3a; Bonferroni tests on log transformed data: auto f/2 vs. auto N/20: $p = 0.093$, auto f/2 vs. auto P/20: $p = 0.077$). Comparisons between the two nutrient-limited diets of the different trophic modes did not reveal significantly different ingestion rates on depleted autotrophs vs. mixotrophs (Fig. 3a; Bonferroni tests: auto N/20 vs. mixo N/20: $p > 0.9$, auto P/20 vs. mixo P/20: $p = 0.418$). The feeding rates of *C. typicus* expressed in C showed also some differences between the balanced (f/2) *K. veneficum* and the nutrient-limited autotrophic diets, but they proved to be statistically significant only for N/20 (Fig. 3b; Bonferroni tests: auto f/2 vs. auto N/20: $p = 0.016$, auto f/2 vs. auto P/20: $p = 0.196$). Higher predation rates, however, were observed when the mixotrophic *K. veneficum* grown in N- and P-depletion were compared to their autotrophic counterparts (Fig. 3b; Bonferroni tests: auto N/20 vs. mixo N/20: $p = 0.026$, auto P/20 vs. mixo P/20: $p = 0.003$). The nitrogen intake of *C. typicus* on the autotrophic nutrient-depleted prey was significantly different when comparing N-limited with f/2 treatment (Fig. 3c; Bonferroni tests: auto f/2 vs. auto N/20: $p = 0.012$, auto f/2 vs. auto P/20: $p = 0.528$). Mixotrophic prey overall enhanced the N intake of *C. typicus*, but differences proved significant only for the P-limited treatments (Fig. 3c; Bonferroni tests: auto N/20 vs. mixo N/20: $p = 0.225$, auto P/20 vs. mixo P/20: $p = 0.009$). Finally, the ingestion of P in *C. typicus* was reduced in the autotrophic P/20 treatment, but due to the variability between the f/2 treatment replicates, differences with the autotrophic f/2 diet did not prove significant neither for N nor P (Fig. 3d; Bonferroni tests: auto f/2 vs. auto N/20: $p = 0.282$, auto f/2 vs. auto P/20: $p = 0.092$). When the nutrient-limited mixotrophic and autotrophic diets are compared for both N and P, mixotrophic prey enhanced the intake of P, but again differences were only significant for the N/20 treatment (Fig. 3d; Bonferroni tests: auto N/20 vs. mixo N/20: $p < 0.001$, auto P/20 vs. mixo P/20: $p = 0.213$).

Copepod reproductive rates and gross-growth efficiency

P. grani egg production rate (EPR) was the lowest on the two nutrient-limited autotrophic *K. veneficum* diets and they were both significantly different from the one measured on the diet based on autotrophic *K. veneficum* grown in f/2 medium (Fig. 4a; Bonferroni tests: auto f/2 vs. auto N/20: $p < 0.001$, auto f/2 vs. auto P/20: $p < 0.001$). Feeding on the mixotrophic *K. veneficum* significantly enhanced the EPR of *P. grani* compared to the autotrophic nutrient-limited counterparts (Fig. 4a; Bonferroni tests: auto N/20 vs. mixo N/20: $p < 0.001$, auto P/20 vs. mixo P/20: $p < 0.001$). Hatching success in *P. grani* was generally higher on autotrophic diets

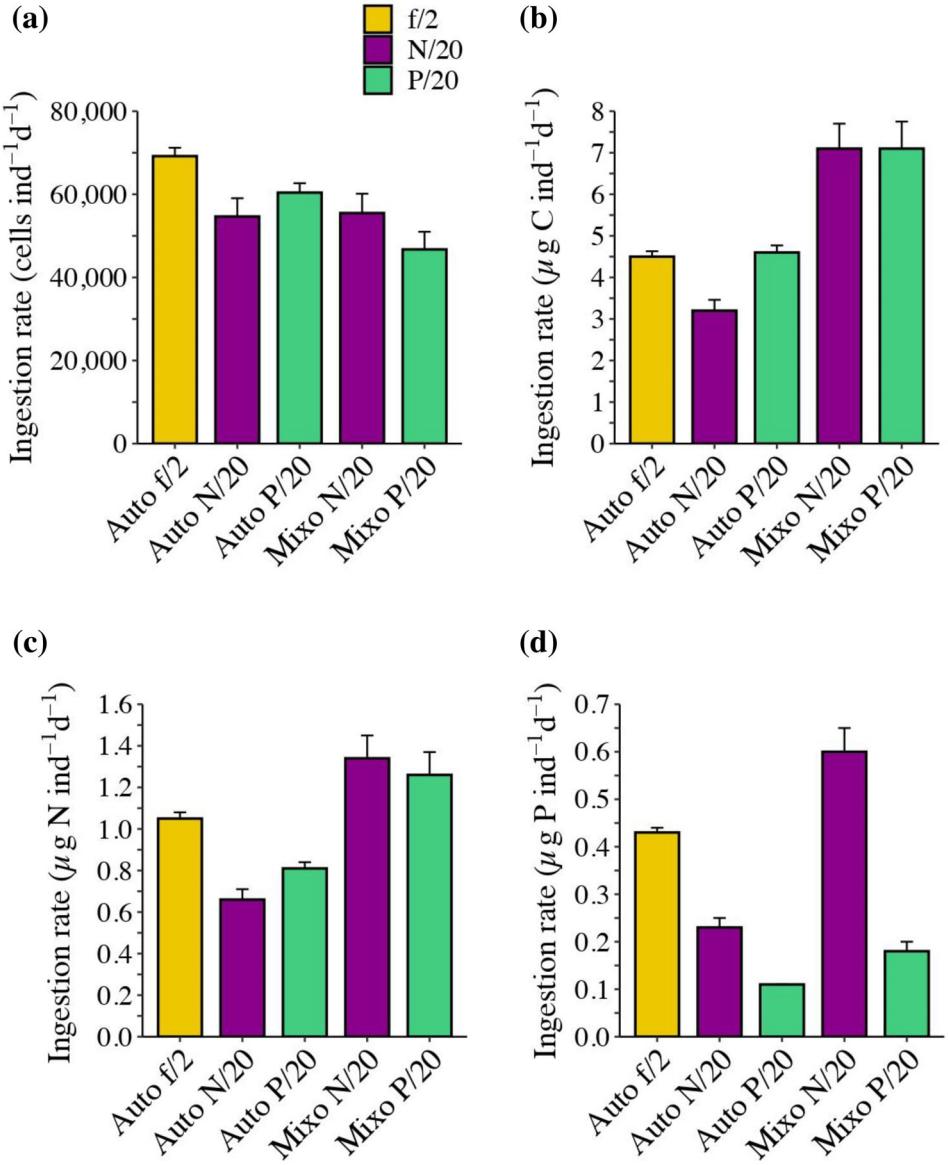

Fig 2. Ingestion rate of the copepod *P. grani* on the prey *K. veneficum* grown under nutrient repletion (f/2) and deficiency (N/20 vs. P/20) and different trophic modes (autotrophic vs. mixotrophic). Ingestion rate is expressed in terms of (**a**) cells, (**b**) carbon, (**c**) nitrogen, and (**d**) phosphorus. Data are average ± SE of the mean.

(on average, 94% ± 1.2%) than on mixotrophic nutrition (on average, 83% ± 0.2%). Differences in hatching measured on the three autotrophic diets were not significant (Fig. 4b; Bonferroni tests: auto f/2 vs. auto N/20: $p = 0.677$, auto f/2 vs. auto P/20: $p = 0.068$), but among the nutrient-limited treatments, significantly different hatching success emerged when autotrophs and mixotrophs were compared (Fig. 4b; Bonferroni tests: auto N/20 vs. mixo N/20: $p = 0.006$, auto P/20 vs. mixo P/20: $p = 0.043$). *P. grani* recruitment rates (combined effect of EPR and hatching success) on the depleted autotrophic diets were significantly lower (30% and 43% reduction on N/20 and P/20 diets, respectively) with respect to *K. veneficum* f/2 (Fig. 4c; Bonferroni tests: auto f/2 vs. auto N/20: $p < 0.001$, auto f/2 vs. auto P/20: $p < 0.001$). Mixotrophic nutrient-limited diets yielded higher recruitment rates in *P. grani* compared to the nutrient-limited autotrophic *K. veneficum* (Fig. 4c; Bonferroni tests: auto N/20 vs. mixo N/20: $p = 0.038$, auto P/20 vs. mixo P/20: $p = 0.003$). Figure 4d shows the *P. grani* C-GGE under the different diets tested. The highest average C-GGE among treatments was close to 0.4. P-limitation reduced significantly C-GGE of *P. grani* fed the autotrophic *K. veneficum* in comparison with balanced f/2 diet, while N-limited diet kept similar values as the autotrophic f/2 one (Fig. 4d; Bonferroni tests: auto f/2 vs. auto N/20:

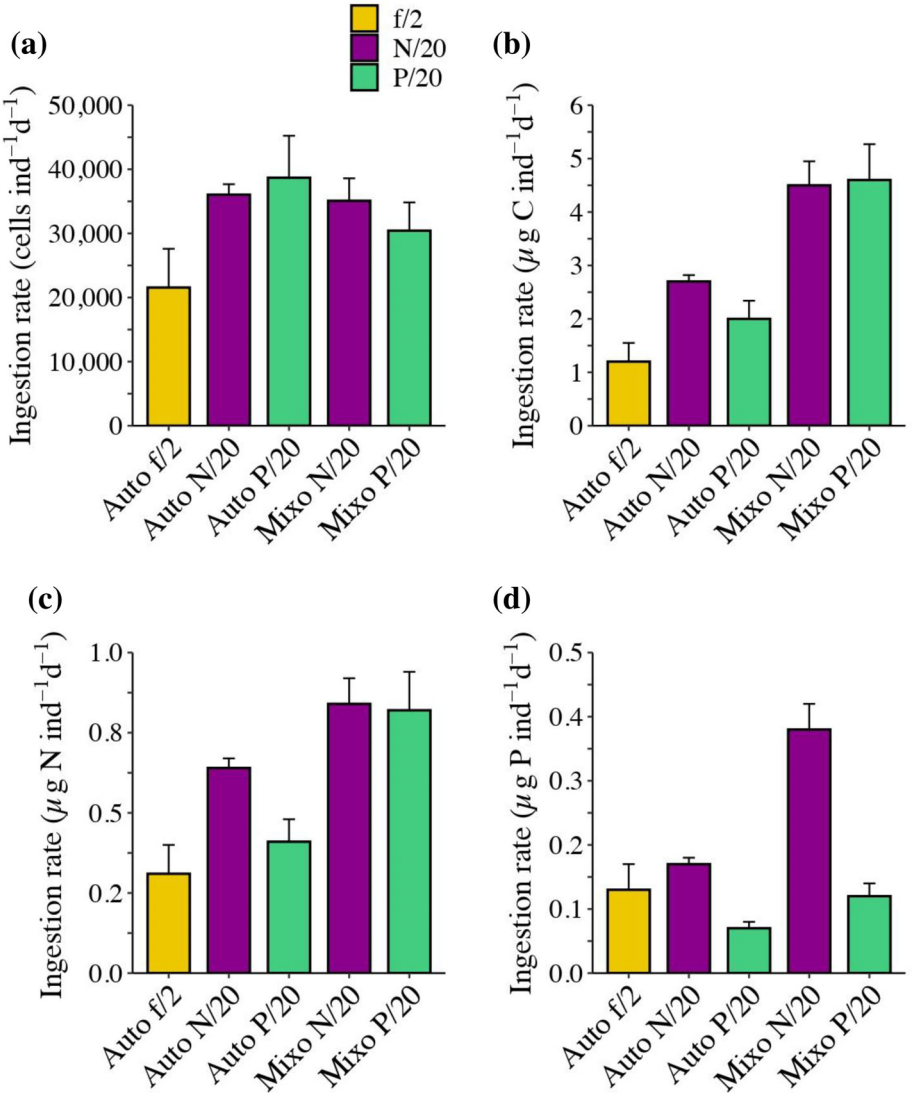

Fig 3. Ingestion rate of the copepod *C. typicus* on the prey *K. veneficum* grown under nutrient repletion (f/2) and deficiency (N/20 vs. P/20) and different trophic modes (autotrophic vs. mixotrophic). Ingestion rate is expressed in terms of (**a**) cells, (**b**) carbon, (**c**) nitrogen, and (**d**) phosphorus. Data are average ± SE of the mean.

$p = 0.452$, auto f/2 vs. auto P/20: $p = 0.007$). The N-limited mixotrophic diet resulted in lower C-GGE than the autotrophic counterpart, whereas both P/20 treatments did not differ (Fig. 4d; Bonferroni tests: auto N/20 vs. mixo N/20: $p < 0.001$, auto P/20 vs. mixo P/20: $p > 0.9$).

The EPR of *C. typicus* was overall much lower than that exhibited by *P. grani*. When comparing the autotrophic dietary treatments, nutrient limitation resulted in somewhat lower EPR than the balanced (f/2) autotrophic diet, but these differences were not statistically significant (Fig. 5a; Bonferroni tests: auto f/2 vs. auto N/20: $p > 0.9$, auto f/2 vs. auto P/20: $p = 0.235$). In P-limited treatments, mixotrophic nutrition enhanced EPR of *C. typicus*, while no variation was observed when comparing N-poor diets between trophic modes (Fig. 5a; Bonferroni tests: auto N/20 vs. mixo N/20: $p > 0.9$, auto P/20 vs. mixo P/20: $p = 0.006$). Overall, in *C. typicus*, nutrient limitation did not result in significantly lower hatching success between the nutrient-limited and nutrient-balanced (f/2) autotrophic *K. veneficum* (Fig. 5b; Bonferroni tests: auto f/2 vs. auto N/20: $p = 0.712$, auto f/2 vs. auto P/20: $p = 0.071$). Contrarily, the N-limited mixotrophic treatment showed significant reduction in the hatching success of *C. typicus* compared to the autotrophic N/20 form, whereas for P the difference was not significant (Fig. 5b; Bonferroni tests: auto N/20 vs. mixo N/20: $p < 0.001$, auto P/20 vs. mixo P/20: $p = 0.162$). Considering solely the autotrophic forms, there was a trend of reduction in the recruitment rate of *C. typicus*, particularly for the P-limited treatment, when compared to

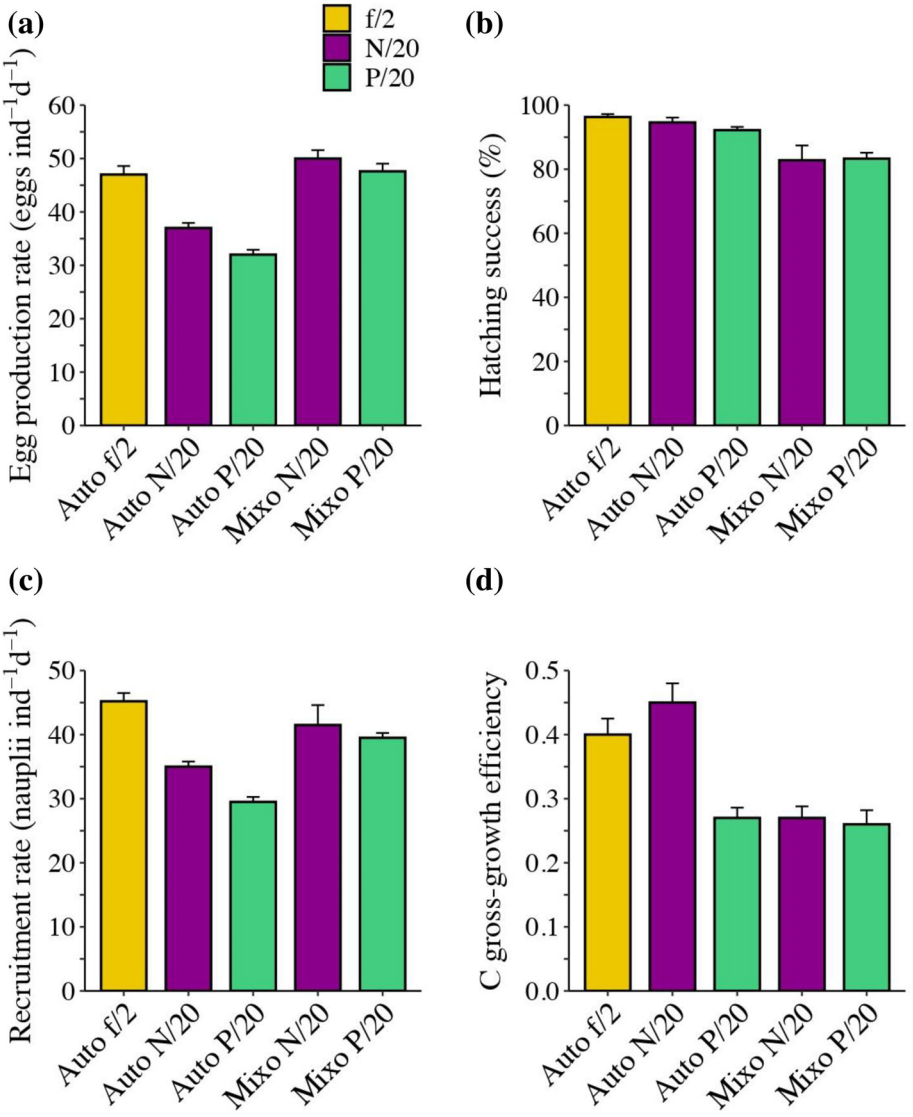

Fig 4. Reproduction and growth in the copepod *P. grani* on the prey *K. veneficum* grown under nutritional repletion and deficiency (N/20 vs. P/20) and different trophic modes (autotrophic vs. mixotrophic). (**a**) Egg production rate, (**b**) egg hatching success, (**c**) recruitment rate, and (**d**) C-gross-growth efficiency. Data are average ± SE of the mean.

K. veneficum f/2, but the differences were not statistically significant (Fig. 5c; Bonferroni tests: auto f/2 vs. auto N/20: $p = 0.809$, auto f/2 vs. auto P/20: $p = 0.143$). The resulting recruitment in the P-limited treatment was enhanced in the mixotrophic vs. the autotrophic counterpart (Fig. 5c; Bonferroni tests: auto N/20 vs. mixo N/20: $p > 0.9$, auto P/20 vs. mixo P/20: $p = 0.012$). Compared to *P. grani*, in *C. typicus* nutrient limitation had an overall major negative impact on C-GGE (Fig. 5d). Substantial reduction in C-GGE was observed on a nutrient-deficient autotrophic diet compared to f/2 *K. veneficum* (Fig. 5d; Bonferroni tests: auto f/2 vs. auto N/20: $p = 0.045$, auto f/2 vs. auto P/20: $p = 0.071$). Similarly, low C-GGE resulted upon consumption of the mixotrophic N- and P-depleted prey. Despite N-limited mixotrophs might have induced a lower C-GGE with respect to N-limited autotrophs, overall, no significant differences emerged from the comparison between nutrient-deplete groups (Fig. 5d; Bonferroni tests on log transformed data: auto N/20 vs. mixo N/20: $p = 0.334$, auto P/20 vs. mixo P/20: $p > 0.9$).

Discussion

Stoichiometric molar ratios are very well accepted proxies of nutritional quality in dietary ecology; these ratios are known to change as function of cell size, light availability, and reliance on external inorganics, ultimately dictating the main metabolic strategy (Sterner and Elser 2008; Ho et al. 2020). At the end of *K. veneficum* growth phase, our

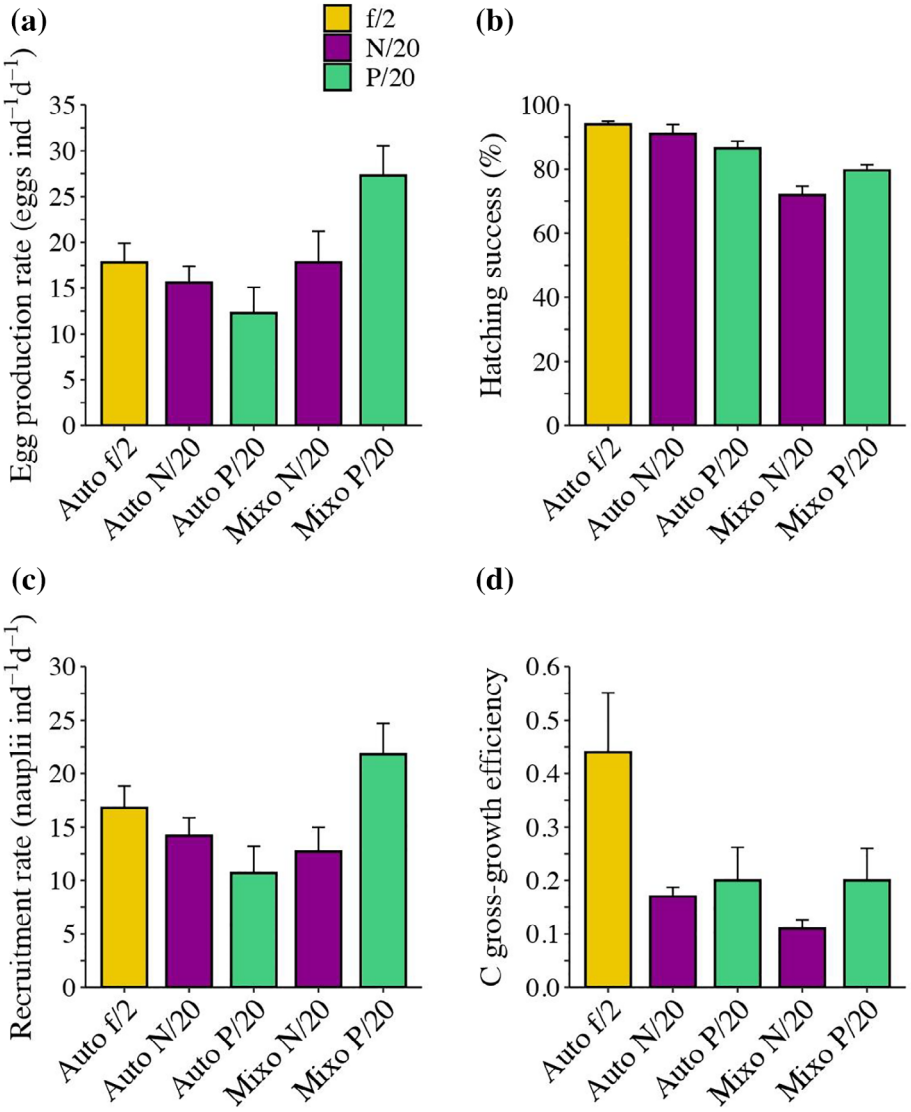

Fig 5. Reproduction and growth in the copepod *C. typicus* on the prey *K. veneficum* grown under nutritional repletion and deficiency (N/20 vs. P/20) and different trophic modes (autotrophic vs. mixotrophic). (**a**) Egg production rate, (**b**) egg hatching success, (**c**) recruitment rate, and (**d**) C-gross-growth efficiency. Data are average ± SE of the mean.

cultures were harvested from extreme inorganic N : P environments (range 0.23–8000 in N and P limitation, respectively), very far from the average seawater N : P ratio (20 : 1) (Redfield 1934; Ryther and Dunstan 1971), which led to adjustments in the stoichiometric composition of the dinoflagellate. As expected, *K. veneficum* grown as pure autotrophs showed large variation in stoichiometry between nutrient treatments; on the contrary, the mixotrophic forms showed an increase in cell size and compensated the nutrient imbalance as they exhibited more stable ratios (C : N) and, under N-depletion, they were more akin to autotrophic *K. veneficum* f/2 (N : P). Generally, P limitation affected the dinoflagellate stoichiometry to a greater extent, whereas limitation by N might have been less severe in our *K. veneficum* cultures. In fact, after the second day from the start of protist conditioning, inorganic P was depleted in P-limited cultures, whereas N exhaustion was observed after 5 d in N-limited treatments (Fig. S1a). Our morpho-stoichiometric observations agree with literature findings regarding the increase in cell size (Ward and Follows 2016; Ho et al. 2020) and stabilization of seston stoichiometry as a consequence of a mixotrophic metabolism (Moorthi et al. 2017). Both these ecological implications may result from the ability of *K. veneficum* to acquire the limiting nutrient from phagotrophy on *R. salina* (Lin et al. 2017), and thus increase in size upon engulfment of the prey. The more pronounced effect of P- vs. N-limitation on cellular stoichiometry could be explained by the ability of several dinoflagellate to use N reserves even in the lack of P (Dagenais-Bellefeuille

and Morse 2013). It is noteworthy to mention that overall, the stoichiometric ratios we found in *K. veneficum* (even for the nutrient-replete treatment) appear rather far from the Redfield values. However, it is known that some dinoflagellates have lower C : P and N : P ratios than the canonical Redfield ratio, and precisely among Gymnodiniales to which *Karlodinium* belongs, C : P and N : P ratios are even lower and very close to the values we found in this study (Carnicer et al. 2021).

When tested under nutrient-imbalanced environments, mixotrophic nutrition resulted in enhanced ingestion in both calanoids compared with autotrophic diets (Figs. 2, 3). The higher nutrient uptake on nutritionally insufficient mixotrophs may be due to the extra nutrient supply acquired by the dinoflagellate from its cryptophyte prey (Li et al. 2000; Adolf et al. 2006). Furthermore, if we consider the elemental content of the *K. veneficum* cultures normalized to cell volume (Table 2), we can clearly see that C, N, and P contents were larger in mixotrophic treatments with respect to autotrophic counterparts grown in similar NP environments. These findings provide experimental evidence of previously formulated in silico hypotheses of enhanced trophic transfer when mixotrophy is accounted in food webs (Ward and Follows 2016; Stoecker et al. 2017). Despite the general pattern of ingestion, the two calanoids exhibited rather different grazing pressure on *K. veneficum*. *P. grani* fed at relatively high rate on the dinoflagellate, whereas *C. typicus* ingestion was more than two-fold lower. Even if the two calanoids under study shared similar body size, they showed distinct physiological response to *K. veneficum*. The ingestion patterns we observed might have been driven by different size and palatability of the *K. veneficum* diets influencing the two predators differently, as evidenced in other studies (Zheng et al. 2011; Xu et al. 2018). It is known that *C. typicus* may show a more carnivorous behavior compared to *Acartia* species (Boersma et al. 2014) and that the lower end of the size optimality spectrum for this copepod species is rather high ($\sim 10\,\mu$m prey diameter) (Calbet et al. 2007). We argue, then, that the comparatively low feeding rates of *C. typicus* upon *K. veneficum* are the result of the small size of the dinoflagellate, which make it less suitable as prey. Moreover, when nutritionally balanced prey are ingested, a lower amount would be enough to satisfy the predator requirements. *C. typicus* might ingest more cells when these are nutrient-poor, a form of compensatory behavior already reported to occur in marine copepods (Augustin and Boersma 2006; Prince et al. 2006; Malzahn and Boersma 2012) and freshwater cladocerans (DeMott et al. 1998; Plath and Boersma 2001).

Overall, the EPR values obtained in our experiments are in agreement with previous field and laboratory studies on *P. grani*, reported to lie between 30 and 70 eggs ind^{-1} d^{-1} (Rodríguez et al. 1995; Isari et al. 2013; Saiz et al. 2015) and *C. typicus*, accounting for 20–60 eggs ind^{-1} d^{-1} (Dagg 1978; Carlotti et al. 1997; Ianora et al. 2007; Saiz et al. 2007). As for ingestion patterns, also the EPR response to *K. veneficum* diets was rather different between the two calanoids under study. Yet, contrarily to *P. grani*, the most likely reason for the low reproductive yield of *C. typicus* in this study may be ascribed to the nutritional inadequacy and suboptimal size of *K. veneficum* as prey (Miralto et al. 1995), as already discussed above. As expected, nutrient limitation in the autotrophic strain of *K. veneficum* resulted in impairment of EPR. Insufficient or stoichiometrically imbalanced microalgal diet can diminish the reproductive physiological performance of copepods (Jones et al. 2002; Arendt et al. 2005; Zheng et al. 2011; Bentley et al. 2021). Generally, in our experiments, reproduction was affected more by P- than N-limitation in both copepod species. P is required for RNA synthesis, membrane structure (Sterner and Elser 2008) and it is particularly important during copepod ontogenetic development (Malzahn and Boersma 2012; Meunier et al. 2016; Saiz et al. 2020). P-deficient *Heterocapsa* sp. drastically reduced the EPR in *P. grani* in comparison to both N-deplete and nutritional (f/2) dinoflagellates to a similar extent as in this study (Isari et al. 2013). P-deficient prey are also known to affect negatively somatic growth of copepods (Malzahn and Boersma 2012). Nevertheless, signs of significant reproductive impairment were also present upon consumption of autotrophic N-limited *K. veneficum* even if to a lesser extent and significantly only in *P. grani*. N is the essential building block for amino acids, proteins, nucleic acids, and chlorophyll (Sterner and Elser 2008). Therefore, a decrease in EPR is also expected and has been previously documented in copepods fed N-depleted diets (Jones et al. 2002; Augustin and Boersma 2006; Isari et al. 2013). Especially for *C. typicus*, the higher cell intake of nutrient-limited autotrophs with respect to nutrient-balanced *K. veneficum* f/2 might not have been enough to buffer the effect of P limitation on EPR we observed. A similar outcome was also reported in *A. tonsa* fed nutritionally insufficient *Karenia brevis*, where a reduced EPR was not balanced out for by the compensatory feeding effort conducted by the copepod (Prince et al. 2006). The decrease in EPR observed on limited autotrophic diets was reverted when copepods fed on limited mixotrophic *K. veneficum*. In fact, the magnitude of enhancement of EPR observed in the N- and P-limited mixotrophic diet reached values similar to the reproductive output when fed the nutritional autotrophic *K. veneficum* f/2 (Figs. 4a, 5a). The compensatory effect of phagotrophic nutrient acquisition operated by the mixoplanktonic *K. veneficum* was hence transferred to copepod level, leading to a recover in the reproductive performance in both limited nutrition types in *P. grani*, whereas *C. typicus* upgrade was only meaningful under P depletion. This evident upgrade is likely conferred by the extra supply of nutrients acquired by *K. veneficum* via phagotrophy on *R. salina*; nutrient-limited mixotrophic *K. veneficum* have higher N and P content than the nutrient-limited autotrophic counterparts (Table 2). Overall, the combined effect of cellular elemental content of the mixotrophic prey and enhanced

grazing effort on them might explain the relationship between EPR, ingestion, and prey morpho-stoichiometric traits.

Hatching did not vary significantly on the basis of nutrient limitation in *P. grani*, but slightly higher hatching (~ 12%) resulted from ingestion of autotrophs vs. mixotrophs. *C. typicus*, instead, suffered a significant hatching decrease on P-limited autotrophic diet compared to P-limited mixotrophs. From recruitment rates, we gather similar conclusion as those already discussed for EPR. Stronger impairment is observed under P starvation in both copepod species and moderate effect under N limitation only emerged in *P. grani*. Beside stoichiometric theories placing single nutrients as determining factors of food quality, there are also other biochemical aspects to consider, such as the content and composition of fatty acids (Tang and Dam 1999; Tang and Taal 2005), known to be crucial in egg development and hatching (Jónasdóttir 1994; Broglio et al. 2003; Arendt et al. 2005). Although we cannot provide fatty acid data in our experiment, it is reasonable to suggest that *K. veneficum* fatty acids may change along with stoichiometry (Klein Breteler et al. 2005; Jónasdóttir 2019) or as function of phagotrophy (Adolf et al. 2007; Calbet et al. 2011).

Our C-GGE calculated for *P grani* fell within the normal range estimated in calanoid copepods (Ikeda 1974) as it was found between 0.26 and 0.45, including the nutrient-balanced autotroph (f/2) accounting for 0.40, whereas C-GGE was found on a mean lower level (0.11–0.20) in *C. typicus* with the exception of the autotrophic f/2 diet (0.44). Hence, overall, *K. veneficum* f/2 induced the highest advantage on the reproductive effort relative to the ingestion in both copepod species. P limitation had stronger declining effect on C-GGE than N limitation in *P. grani*, as previously documented in cladocerans fed a P-deficient diet (DeMott et al. 1998), whereas for *C. typicus* both N and P limitation had a remarkable effect on C-GGE (for P, differences were only significant if one-tailed). Despite the overall beneficial effects of mixotrophic prey for copepod ingestion and reproduction, in terms of C-GGE there was no clear benefit and occasionally resulted in a reduction in comparison to a diet consisting of nutrient-limited autotrophs. This observation does not come as a surprise. In fact, lower C-GGE in *P. grani* fed mixotrophic *K. veneficum* (0.15) in nutrient starvation in comparison to nutrient-depleted (0.29) and replete (0.26) autotrophic cultures had already been reported (Traboni et al. 2020), in agreement with the decrease observed in this study between N-limited mixotrophic and autotrophic treatments in *P. grani* (39%) and *C. typicus* (32%).

In this study, we evaluated the effect of inorganic N and P shortage on the food quality of the mixoplankter *K. veneficum* for the calanoid copepods *P. grani* and *C. typicus*. Our aim was to test the differential response of copepods to either nutrient depletion and to ascertain whether the metabolic strategy of the dinoflagellate could influence the nutritional value for the predators under these selective oligotrophic circumstances. This is the first experimental evidence to demonstrate that food upgrading achieved via mixotrophy in protists promotes higher nutrient transfer to higher trophic levels represented by copepods. Our results confirm the hypothesis that *K. veneficum* upgrades food quality for calanoid copepods. Mixotrophy enables the dinoflagellate to overcome inorganic limitation of N and P by acquiring the limiting nutrient via the ingestion of prey, which is a strategy adopted by mixoplankton to increase adaptability or persistence in a wider range of ecosystems compared to pure autotrophs (Burkholder et al. 2008). Also, we cannot rule out the role of specific biochemical compounds produced or lacking in protists grown under a given trophic mode may have important effects on copepod reproductive success. The combination of photosynthetic and phagotrophic metabolisms in nutrient-deficient mixotrophs upgrades food quality for copepods *P. grani* and *C. typicus*, leading to enhanced ingestion, reproduction, and overall next-generation recruitment compared to limited autotrophic diets. In ecological terms, our findings support previous hypotheses (Mitra et al. 2014; Ward and Follows 2016; Stoecker et al. 2017; Livanou et al. 2021) that mixotrophy has the potential to enhance nutrient transfer and fuel the biological carbon pump, thus it is worth considering in the assessment of nutrient cycling and food-web dynamics.

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Conflict of Interest

None declared.

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