Ocean acidification exacerbates the effects of paralytic shellfish toxins on the fitness of the edible mussel *Mytilus chilensis*

Carla Mellado, Oscar R. Chaparro, Cristian Duarte, Paola A. Villanueva, Alejandro Ortiz, Nelson Valdivia, Rodrigo Torres, Jorge M. Navarro

**Highlights**
- The association between pCO2 - PST impacts negatively of the physiology of *M. chilensis*
- The association between pCO2 and PST may also result in indirect effect on mussel fitness.
- The inhibition of energy acquisition by PST may negatively impact mussel fitness.

**Graphical Abstract**
![Graphical Abstract](image)

**Abstract**
High latitudes are considered particularly vulnerable to ocean acidification, since they are naturally low in carbonate ions. The edible mussel *Mytilus chilensis* is a common calcifier inhabiting marine ecosystems of the southern Chile, where culturing of this species is concentrated and where algal blooms produced by the toxic dinoflagellate *Alexandrium catenella* are becoming more frequent. Juvenile *Mytilus chilensis* were exposed to experimental conditions simulating two environmental phenomena: pCO2 increase and the presence of paralytic shellfish toxins (PST) produced by the dinoflagellate *Alexandrium catenella*. Individuals were exposed to two levels of pCO2: 380 μatm (control condition) and 1000 μatm (future conditions) over a period of 39 days (acclimation), followed by another period of 40 days exposure to a combination of pCO2 and PST. Both factors significantly affected most of the physiological variables measured (feeding, metabolism and scope for growth). However, these effects greatly varied over time, which can be explained by the high individual variability described for mussels exposed to different environmental conditions. Absorption efficiency was not affected by the independent effect of the toxic diet; however, the diet and pCO2 interaction affected it significantly. The inhibition of the physiological processes related with energy acquisition by diets containing PST, may negatively impact mussel fitness, which could have important consequences for both wild and cultured mussel populations, and thus, for socioeconomic development in southern Chile.

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**Keywords:** Physiological energetics, PST, CO2, Mussels, Toxic dinoflagellates, Climate change.
This study evaluates the combined effects of two environmental drivers that could occur simultaneously in southern Chile, namely a pCO2 increase (associated with global climate change) and the presence of PST, on the physiological performance (feeding, metabolism and growth) of juvenile M. chilensis. We hypothesize that the combined effects of these two factors decreases the fitness of M. chilensis expressed in terms of the energy available for growth processes (scope for growth).

2. Materials and methods

2.1. Collection and acclimation of mussels

Mussels (Mytilus chilensis) were collected (May 2012) from suspended cultures in Huelmo Bay, Puerto Montt, southern Chile (41°40′ S, 73°02′ W) and transported to the laboratory under controlled temperature conditions. Specimens with a shell length range of 2.4–2.9 cm were selected; these were all juveniles in the sense that they were not yet capable of reproduction. In preparation for physiological measurements, mussels were divided into two groups and acclimated for 39 days in 20 aquaria (4 L), each containing 9 individuals, one group exposed to 380 μatm pCO2 (control), the other to 1000 μatm pCO2 (predicted future condition, IPCC, 2013). Temperature was maintained at 14 °C by partially immersing the aquaria in thermostatically controlled water baths. Salinity was maintained at 30, and all experimental mussels were fed continuously (peristaltic pump) with the microalga Isochrysis galbana (ca 1.5 mg L⁻¹). Seawater was changed daily with water pre-equilibrated to the above conditions.

2.2. Diets and experimental design

The dinoflagellate Alexandrium catenella (strain ACC02) was cultured in filtered seawater (0.45 μm) at 14 °C and a 14:10 photoperiod (light: dark), using L1 culture medium (Guillard, 1975). According to Velásquez and Navarro (2014), strain ACC02 has an average toxin concentration of 10.3 ± 0.91 fmolores STX eq. cell⁻¹. The microalga Isochrysis galbana was cultured in filtered seawater (0.45 μm) at 25 °C and a 14:10 photoperiod (light: dark), using f/2 culture medium (Guillard, 1975).

Following 39 days of acclimation to the two pCO2 treatments, mussels were exposed for 40 days to two combinations of pCO2 and PST-containing diets. For each treatment (380 μatm pCO2 + control diet; 800 μatm pCO2 + toxic diet; 1000 μatm pCO2 + control diet; 1000 μatm pCO2 + toxic diet) 5 mussels were placed in each of 5 replicate aquaria. The control diet was 100% L. galbana, and the toxic diet consisted of 70% A. catenella and 30% L. galbana (by weight). Both diets were delivered continuously through a peristaltic pump (Masterflex 7524). In all treatments the diet offered represented a daily contribution of 4% of the soft tissue weight of the mussel biomass in the tank, calculated as follows:

Mean dry weight of a mussel = 130 mg; 4% dry weight = 5.2 mg
Dry weight of 10⁶ A. catenella cells = 6.19 mg
Dry weight of 10⁶ L. galbana cells = 0.032 mg
Weight of A. catenella cells added per day per mussel = 0.7 × 5.2 mg = 3.64 mg (≈ 0.59 × 10⁶ cells)
Weight of L. galbana cells added per day per mussel = 0.3 × 5.2 mg = 1.56 mg (≈ 49.1 × 10⁶ cells)

Seawater in the mussel holding tanks was replaced daily by gradual introduction of water from the equilibration tanks (see below), maintaining the appropriate diet and pCO2 levels.
2.3. Seawater equilibrium system

Three polyethylene header tanks (250 L, referred to here as equili-
bration tanks) were filled with filtered (1 μm) seawater taken from
the subtidal zone at Calafuco (39°44′S, 73°23′W) and adjusted to two
pCO2 levels, current atmospheric levels (approx. 380 ppm) and the
worst case scenario for the end of the century (approx. 1000 ppm)
by bubbling air or an air–CO2 mixture (Torres et al., 2013). During the
experiments, water pH and total alkalinity were monitored in each tank
every 3 d. All pH measurements were done in a closed 25 mL thermo-
static cell at 25.0 °C with a Metrohm 713 pH meter (input resistance
>1013 Ω, 0.1 mV sensitivity and nominal resolution 0.001 pH units)
and a glass combined double junction Ag/AgCl electrode (Metrohm
model 6.0219.100) calibrated with 8.089 Tris buffer at 25 °C; pH values
are reported using the total hydrogen ion scale (DOE, 1994). The overall
uncertainty in the measured pH values was estimated by Torres et al.
(1999) as 0.006 pH for surface waters (pH near 8) and <0.009 pH for
very acid waters (pH 7.2). Temperature and salinity were measured in
the equilibration tanks with a small conductivity-temperature-depth
instrument (Ocean Seven 305 Plus CTD, www.idronaut.it). Total Alka-
linity (AT) was determined in seawater samples by automatic titration
with HCl (Haraldsson et al., 1997) using CRM from Andrew Dickson Lab-
oratory to constrain analytical uncertainties; Based in the results of
2017 Inter-laboratory Comparison of CO2 Measurements (coordinated.
Emily Bockmon and Andrew Dickson, unpublished data) we calculate
that the difference between our analysis (Carbonate System Laboratory
at CIEP) and Scripps Institution of Oceanography (CRM Batches 162 and
164) were approximately 0.1% which is considerable adequate
(Bockmon and Dickson, 2015).

The pH, total alkalinity (AT), phosphate (Strickland and Parsons,
1972), dissolved silicate (Strickland and Parsons, 1972), and hydro-
graphic data were used to calculate the remaining carbonate system pa-
rameters and the saturation state of seawater with respect to aragonite
and calcite using CO2SYS software (Lewis and Wallace, 1998), set with
Mehrbach solubility constants (Mehrbach et al., 1973), as refitted
by Dickson and Millero (1987).

2.4. Measurement of physiological variables

On each sampling date (days 40, 41, 50, 60, and 80) one mussel was
taken at random from each replicate aquarium for each treatment, i.e. 5
mussels per treatment, for physiological measurements. Mussels
reached a PST concentration of 128.0 μg STXeq./100 g tissue at the end
of the experiment at 380 μatm CO2 and 85.6 μg STXeq./100 g tissue at
1000 μatm CO2.

Clearance rate (CR) was measured in a static system in which the de-
crease in particle concentration in each experimental chamber resulting
due to feeding activity was monitored periodically (Widdows, 1985).
Before the measurement period, the experimental specimens were
allowed to acclimate for 30 min in the chamber, after which the algal
cells constituting the appropriate diet (1.5 mg L−1) were added. All ex-
periments were conducted at 14 °C and salinity 30. Each experimental
mussel was placed in an experimental chamber (0.5 L), one mussel
per chamber, and the decrease in the number of particles was measured
after 30 min using a particle counter (Beckman Z2) fitted with a 100 μm
aperture tube. The consumed cells were then replaced to reset the cell
concentration to the initial value, and the clearance rate (L h−1)
for the 30 min period calculated (Coughlan, 1969). This procedure was
repeated 7 times and a mean value of clearance rate determined,
representing an overall clearance rate over a period of approximately
4 h. At no time was the particle concentration in the chamber allowed
to fall below 60% of the initial value. No pseudofaeces were produced
with the food concentration used. During each set of measurements, a
chamber without mussels was used as a control for each treatment to
account for natural changes in particle concentration (growth or algae
sedimentation). The organic ingestion rate was calculated as the prod-
uct of clearance rate and the organic content of the diet.

Absorption efficiency (AE) was estimated by the ratio method of
Conover (1966). Faeces were collected from each experimental mussel
after clearance rate measurements were completed. Samples of food
and faeces were retained on pre-ashed, pre-weighted Whatman GF/C fil-
ters (1.2 μm pore size), rinsed with ammonium formate (3%), dried
to constant weight at 100 °C, weighed, combusted at 450 °C for 3 h in
a muffle furnace and weighed again to determine the organic and inor-
ganic fractions. Absorption rate was calculated as the product of organic
ingestion rate and absorption efficiency.

Immediately after the clearance rate measurements were made, am-
monia excretion (VNH4-N) and oxygen uptake (VO2) were determined
on the same individuals used for clearance rate determinations. Individ-
ual mussels were placed in sealed glass beakers (0.13 L) containing fil-
tered (0.45 μm) seawater. During each measurement set, one
additional beaker containing filtered seawater, but without mussels, was
used as a control. After 2 h, water samples (5 mL) were taken from
each beaker for the determination of ammonia excretion using the
colorimetric method of Solórzano (1969) as modified by Widdows
(1985). Oxygen concentration was determined by the micro-Winkler ti-
tration method (Ohle, 1953) and the oxygen uptake by each mussel was
calculated as the difference between the oxygen content of the water in
the control and experimental beakers over a period of 2 h.

2.5. Scope for Growth (SGF)

Measurements of the energy available for growth, termed scope for
growth (SGF), provide a rapid and quantitative assessment of the en-
ergy status of the bivalve (Widdows, 1985). Scope for growth was calcu-
lated after converting VO2, VNH4-N and absorbed organic matter from
the diet to energy equivalents (J · h−1). 1 μL O2 = 19.9 J; 1 μg
NH4-N = 0.0249 J (Elliot and Davison, 1975) and 1 mg of organic ma-
terial from the diet = 21 J (McLusky, 1989).

2.6. Statistical analyses

The prediction that the combined effects of pCO2 and PST decreases
the fitness of M. chilensis expressed in terms of energy acquisition was
tested by means of random-intercept linear mixed models (LMM) com-
puted separately for each dependent variable. The models included diet,
time, and pCO2 as fixed and fully crossed factors, and the identification
of each aquarium as a random factor influencing the intercept (i.e. base-
line level) of the model. The aquarium was included as random factor in
order to account for the repeated measurements conducted over time.
In addition, we modelled the temporal autocorrelation of errors as an
autoregressive (AR1) process. The effects parameters were estimated
by means of maximum likelihood. Residual-by-quantile, density, and
residual-by-fitted value plots were graphically inspected as model diag-
nostics. Moreover, we assessed the degree of autocorrelation of errors
by means of Auto-Correlation Function (ACF) plots. These plots showed
minimum degrees of autocorrelation of residuals, always r < 0.2 (Pear-
son Product Moment correlation). Marginal and conditional coefficients
determination (pseudo-R2) were computed as estimators of variance
accounted for the fixed factors and the entire model, respectively
(Nakagawa and Schielzeth, 2013). Each LMM was further analysed by
means of a Wald-test analysis of variance (ANOVA). After a signi-
cant interactions, the paired
comparisons were conducted for a given factor within each level of
the other factor. In the cases of statistically significant interactions, the paired
comparisons were conducted for a given factor within each level of
the other factor involved in the interaction. Tukey’s multiplicity adjust-
ment was used to reduce the Type-I error rate. All statistical analyses
were conducted in the R programming environment version 3.5.0
(R Core Team, 2018) – the ggplot2, dplyr, nime, MuMIn, and emmeans R
packages were required to complete the statistical analyses.
3. Results

3.1. Experimental seawater system

The physical and chemical seawater characteristics are detailed in Table 1, and were previously described in a parallel study on seawater calcification (Duarte et al., 2014). Values for salinity were very similar at both 380 and 1000 µatm pCO2. The pH, carbonate ion concentration and the saturation state of seawater with respect to aragonite decreases with increasing pCO2.

3.2. Physiological parameters

3.2.1. Clearance rate

The effect of the toxic diet on clearance rate was negative and dependent on the sampling time, these effects remained relatively similar between pCO2 treatments (Fig. 1). The ANOVA (Wald test) conducted on the linear mixed model (LMM) showed a statistically significant interaction between diet and time (Table 2). This interaction was given by the statistically significant and negative effects of the toxic diet that were observed at days 40 and 41 (−0.61 ± 0.07 L h⁻¹ and −0.26 ± 0.07 L h⁻¹, respectively, mean [standard error of the mean]); but that became non-significant after 60 days (Table S1). The fixed factors in the LMM accounted for ca. 56% of the variation in clearance rate; the entire model explained ca. 62% of the variation in this variable.

3.2.2. Absorption efficiency

Absorption efficiency was dependent on sampling time, diet, and pCO2 (Fig. 2). This observation was supported by the LMM and ANOVA, which showed a statistically significant interaction between the three factors (Table 1). According to this second-order interaction, we observed statistically significant effects of the toxin at days 40 and 60 on the absorption efficiency of the mussels exposed to 380 µatm (−9.2 ± 4.04 % and 16.5 ± 4.04 %), respectively (Table S1). On the other side, we detected statistically significant effects of the toxic diet at days 50 and 80 (−14.1 ± 4.04 % and −11.1 ± 4.04 %, respectively) of mussels exposed to 1000 µatm (Table S1). The fixed factors accounted for ca. 71% of the variation in absorption efficiency. The same proportion of variance was explained by the entire model, suggesting a negligible effect of sampling units on the variable of interest.

![Image](image-url)

**Fig. 1.** Clearance rate in *Mytilus chilensis* juveniles exposed to toxic (PST) and control diets at 380 (a) and 1000 (b) µatm pCO2. Values represent mean ± standard error.

### Table 1

| Characteristics (mean ± SD) of seawater used to maintain *Mytilus chilensis* individuals during the experimental period in the 250 L tanks equilibrated with air (approx. 380 µatm pCO2) or air – CO2 mixture (approx. 1000 µatm pCO2); TA = total alkalinity; [CO3²⁻] = carbonate ion concentration; Ωar = aragonite; Ωcal = calcite. From Duarte et al. (2014). |
|------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| pCO2 in situ (µatm)          | Experimental pCO2 levels (µatm) | 380              | 1000              |
| pH in situ (pH units)        | 8.06 ± 0.03        | 7.67 ± 0.03       |
| Salinity (psu)               | 31.79 ± 1.88       | 31.87 ± 1.87      |
| TA (µmol kg⁻¹)               | 2156 ± 86          | 2142 ± 93         |
| [CO3²⁻] in situ (µmol kg⁻¹)  | 129 ± 14           | 58 ± 7            |
| Ωar                          | 3.1 ± 0.3          | 1.4 ± 0.2         |
| Ωcal                         | 2.0 ± 0.2          | 0.9 ± 0.1         |

### Table 2

Summary of ANOVA on six physiological variables in mussels exposed to two levels of diet (controls or toxic), pCO2 (380 and 1000), and sampled over time of exposure (after 40, 41, 50, 60, and 80 days) in a fully orthogonal experiment. Degrees of freedom (DF) are provided for the numerator and denominator (num and den, respectively) of each F-ratio.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>Clearance rate</th>
<th>Absorption efficiency</th>
<th>Absorption rate</th>
<th>F</th>
<th>p</th>
<th>F</th>
<th>p</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
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<td>3.21</td>
<td>0.09</td>
<td>0.17</td>
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<tr>
<td>Time</td>
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<td>1.387</td>
<td>0.248</td>
<td>8.02</td>
<td>0.01</td>
<td>5.92</td>
<td>0.00</td>
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<tr>
<td>pCO2</td>
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<td>0.627</td>
<td>46.64</td>
<td>0.00</td>
<td>9.55</td>
<td>0.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet:Time</td>
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<td>0.001</td>
<td>2.72</td>
<td>0.04</td>
<td>3.46</td>
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<tr>
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<td>4.47</td>
<td>0.01</td>
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<table>
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<tr>
<th>Source of variation</th>
<th>DF</th>
<th>Ammonia excretion</th>
<th>Oxygen consumption</th>
<th>Scope for growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td>1</td>
<td>16</td>
<td>6.25</td>
<td>0.02</td>
</tr>
<tr>
<td>Time</td>
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<td>64</td>
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</table>
3.2.3. Absorption rate

This variable showed temporal patterns that depend on the diet and pHCO2 treatments. Moreover, the effects of pHCO2 and diet on absorption rate were interdependent (Table 1; Fig. 3). First, the temporal variation in the effects of the toxin was due to the significant decrease (−0.21 [0.06] mg h⁻¹, averaging across pHCO2 treatments) detected at day 50 of exposure; no other statistically significant effect of diet was observed (Table S1). Second, the time-dependent effect of pHCO2 was supported by an initial increase in absorption rate (0.44 [0.07] mg h⁻¹ day 40) and a subsequent decrease (−0.44 [0.07] mg h⁻¹ day 41) in this variable as a result of the treatment (Table S1). Finally, and averaging across sampling times, the diet by pHCO2 interaction was explained by contrasting effects of the diet under 380 μatm (non-significant) and 1000 μatm (negative) on absorption rate (Table S1). The fixed factors of the LMM explained a 62% of the variation in absorption rate. The entire model accounted for the same proportion of variation.

3.2.4. Ammonia excretion

The temporal patterns of ammonia excretion varied between diet treatments and between pHCO2 treatments (Table 1; Fig. 4). Similar to absorption rate, the diet and pHCO2 treatments interactively affected ammonia excretion (Table 1). A statistically significant diet by time interaction was supported by the significant increase in ammonia excretion observed at day 40 of experimentation (10.4 [4.2] μg N H4-N h⁻¹) averaged across diet treatments (Table S1). Non-significant decreases were observed at days 41 and 50, and significant decreases observed
at days 60 and 80 (−12.6 [4.2] and −14.7 [4.2] μgN H4-N h−1, respectively) (Table S1). The temporal variation in the responses to pCO2 was due to the fact that the marginal effect (i.e. averaged across diets) of this treatment was observed only at day 80 of exposure (−10.2 [4.2] μgN H4-N h−1) (Table S1). Lastly, we detected a pCO2-dependent switch in the response of ammonia excretion to the diet: averaged across times, the toxic diet had a significantly positive effect on the excretion of the mussels exposed to 380 μatm, but a negative effect on those exposed to 1000 μatm (7.6 [2.9] and −18.2 [2.9] μgN H4-N h−1, respectively) (Table S1). The fixed factors in this model accounted for a 60% of the variation in ammonia excretion, whereas the entire model accounted for ca. 62% of the variation in this response.

### 3.2.5. Oxygen consumption

Oxygen consumption varied over time, depending on the exposure to toxic diet and also on the pCO2 treatment (Table 1; Fig. 5). Averaged across pCO2 treatments, the marginal effect of the toxic diet was statistically significant at days 40 and 80 of exposure (0.01 [0.006] and −0.02 [0.006] mL h−1, respectively) (Table S1). On the other side, and averaging across diet treatments, the marginal effect of pCO2 changed from non-significant (until 50 days) to significantly negative at days 60 and 80 of exposure to the enhanced pCO2 (−0.04 [0.006] and −0.02 [0.006] mL h−1, respectively; Table S1). Diet and pCO2, as fixed factors, explained the 57% of the variation in oxygen consumption, and the entire model explained the 69% of the variation in this metric.
3.2.6. Scope for growth

Scope for growth exhibited variable pattern over time, which was dependent on the pCO2 treatments (Table 1; Fig. 6). This interaction was caused by the significant and positive effect on scope for growth detected at day 40, which switched to negative at day 41 (7.95 ± 1.2 and −9.14 ± 1.2 J h−1, respectively; Table S1), and then increases remained statistically non-significant until the end of the experiment (Table S1). The diet and pCO2 treatments interactively affected scope for growth (Table 1). The statistical model explained ca. 61% of the variation in scope for growth (both, fixed factors and entire model).

4. Discussion

Various physiological responses have been described in marine invertebrates to a pCO2 increase, either alone or in combination with other environmental drivers (Fernández-Reiriz et al., 2012; Le Moullac et al., 2016; Wang et al., 2015), but there are no published studies of the combined effects of PST and high pCO2 on the physiology of suspension-feeding bivalves. Wang et al. (2015) found that increasing temperature significantly reduced clearance rate of the mussel Mytilus coruscus, but elevated levels of pCO2 together with a temperature increase did not show a combined effect. This is consistent with Range et al. (2014), who observed that clearance rate in Mytilus galloprovincialis was unaffected by a pCO2 increase. Fernández-Reiriz et al. (2012) showed that observed seawater pCO2 (pH reduced by 0.3 and 0.6 units, relative to the natural pH levels of Ria Formosa lagoon) had no effect on feeding (clearance and ingestion rates) in M. galloprovincialis juveniles. Similarly, in our study pCO2 did not affect clearance rate during the acclimation (39 days) and the experimental period (from day 40 to 80). Other studies have described negative effects of high pCO2 levels on clearance rate in bivalves. Navarro et al. (2016a) found that clearance rate of M. chilensis was significantly affected by pCO2 and temperature, but without interactions between these drivers. Fernández-Reiriz et al. (2011) observed a reduction in feeding in the clam Ruditapes decussatus at the highest experimental pCO2 used (3702 μatm). Similarly, Xu et al. (2016) recorded significantly lower feeding values in R. philippinarum exposed to high pCO2 levels than in clams at ambient pCO2.

The variable physiological responses of marine invertebrates to changes in pCO2 levels described in the literature are probably attributable to the wide range of natural environmental conditions that individuals are exposed in their habitats. Since organisms live periods or its entire life at high pCO2 levels, they have to develop tolerance to pCO2 variability (Widdicombe et al., 2009) or adequately synchronize its development with the natural variability of pCO2. According to Lardies et al. (2014), phenotypic adaptation to environmental fluctuations frequently occurs as a result of pre-existing plasticity, whose role as a major component of variation in physiological diversity is generally recognized. In the case of our study, the experimental mussels were collected from Huelmo Bay, 50 km west of Reloncaví Sound, an area characterized by events of salinity drops which decreases Omega and eventually turning estuarine water undersaturated with respect to calcium carbonate during the cold and rainy winter season (Alarcón et al., 2015). During the warm period phytoplankton productivity cause a seasonal drop in surface water pCO2 (Torres et al., 2011) resulting in highly supersaturated surface water with respect calcium carbonate. Most of the juvenile mussels development takes place under condition of high temperature, omega and food availability (phytoplankton). However, intraseasonal and interannual climatic and oceanographic variability processes are well known modulator this seasonal pattern (e.g. climatic anomalies as rainy/dry periods or changes in meteorological or hydrographical processes that control the mixing and advection of fresh water in the estuary) changing the timing, intensity or synchronization between low salinity/temperature rise/food availability. The adaptation to such environmental variability could partially explain the lack of statistically significant differences in some physiological responses at contrasting pCO2 levels. The significant interaction between diet and time was related to the initial negative effect and the subsequent recovery of the clearance rate, behavior that has been described for different species of bivalves exposed to diets containing PST (Navarro and Contreras, 2010; Wildish et al., 1998). After one day of exposure to the toxic diet (day 41), M. chilensis showed a recovery in feeding activity similar to that described for juveniles and adults of the same species fed with Alexandrium catenella (Navarro et al., 2011; Velásquez and Navarro, 2014). Conversely, oysters, clams, and scallops are highly sensitive to PST toxins, which induce a reduction in suspension-feeding (Bricelj et al., 1996; Gainey and Shumway, 1988b; Lassus et al., 2004; Navarro et al., 2016b). However, the mussel Perna canaliculus showed no reduction in clearance rate when exposed to the toxic dinoflagellate Alexandrium tamarense for four days (Contreras et al., 2012). There is considerable evidence that the feeding

![Fig. 6. Scope for growth in Mytilus chilensis juveniles exposed to toxic (PST) and control diets at 380 (a) and 1000 (b) μatm pCO2. Values represent mean ± standard error.](image)
response of marine bivalves to toxic dinoflagellates, be it inhibition, an increase, or no effect, is species-specific (Bricejel et al., 2005; Contreras et al., 2012; Hégaret et al., 2007; Levereone et al., 2007).

Our data showed that the factor pCO2 affected significantly absorption efficiency in M. chilensis. Similarly, Navarro et al. (2013, 2016a) reported that elevated pCO2 levels (1000, 1200 μatm) significantly reduced absorption efficiency in M. chilensis, suggesting deficiencies in the functioning of the digestive system under hypercapnia. Le Moullac et al. (2016) found that absorption efficiency in the oyster Pinctada margaritifera showed no significant differences in response to different levels of pCO2 (426, 1198, and 3667 μatm pCO2), temperature (22, 26, 30, and 34 °C) or the interaction between the two factors. Fernández-Reiriz et al. (2012) found a significant increase in absorption efficiency in M. galloprovincialis caused by elevated pCO2 which may be related to the optimization of certain digestive enzymes (amylase, glucosidase, and peptidase) under conditions of reduced pH (Areekijseree et al., 2004; Wojtowicz, 1972), facilitating nutrient absorption. In our study, the absorption efficiency was not affected by the treatment of the toxic diet independently, however the interaction diet and pCO2 affected significantly absorption efficiency. Navarro and Contreras (2010) observed a decrease in absorption efficiency in a different population of the same species during the first days of exposure to a diet containing 50% A. catenella. Fernández-Reiriz et al. (2013) found a significant reduction in absorption efficiency when razor clams (Tagelus dombeii) from a non-PST exposure field site were fed a diet containing PST. Absorption rate was also dependent of the interaction pCO2 and diet, with significant lower values in mussels fed toxic diet at the higher level of pCO2 (1000 μatm), but not significant different values were observed at the control level of pCO2 (380 μatm).

It has been described that ammonia excretion increased significantly at high pCO2 in M. galloprovincialis and R. philippinarum, as a result of the enhancement of protein metabolism which contributed to intracellular pH regulation (Fernández-Reiriz et al., 2012; Michaelidis et al., 2005; Xu et al., 2016). Regarding paralytic shellfish toxin (PST), a significant effect on the excretion rate of M. chilensis has also been described by Navarro and Contreras (2010), who associated this response with the capacity of the mussels to degrade the PST toxin, which is a rich source of nitrogen (Pérez, 1998). The present study shows that excretion rate was not affected by high pCO2 in M. chilensis. However, the interaction between diet and pCO2, diet and time and time and pCO2 affected significantly the ammonia excretion of M. chilensis, showing the importance to include not only a single driver, but multiple drivers on the climate change studies (Boyd et al., 2018).

Different responses in oxygen uptake have been described in various bivalves exposed separately to high pCO2 and diets containing PST. Fernández-Reiriz et al. (2012) found that acidified seawater (ΔpH = −0.6) had no effect on oxygen uptake in M. galloprovincialis juveniles. In contrast, Navarro et al. (2016a) described a decrease in oxygen uptake when M. chilensis was exposed to high pCO2, similar to Wang et al. (2015), who reported a significant effect of pH, temperature, and their interaction on oxygen uptake by M. coruscus. Our results showed that oxygen consumption was affected by pCO2 and also by the interactions between pCO2 and diet and time and diet, suggesting a more complex response of M. chilensis when exposed to more than a single environmental driver. Shumway et al. (1985) found a reduction in oxygen uptake in the giant scallop Placopecten magellanicus and the clam Spisula solidissima exposed to diets containing PST, even when there was no decrease in suspension-feeding activity. In contrast, the presence of STX in the diet and its accumulation in the tissues of M. chilensis did not affect oxygen uptake in the present study and also according Navarro and Contreras (2010) and Navarro et al. (2014) for individuals from two populations of the clam Tagelus dombeii which were exposed to similar concentrations of A. catenella (ca. 210.000 cells L−1).

Although scope for growth in M. chilensis was positive for each combination of pCO2 and PST, the diet and pCO2 treatments interactively affected scope for growth, reducing significantly the amount of energy allocated to growth. Other authors have obtained similar results for other bivalve species when considering only pCO2 increases, e.g. the oyster Pinctada margaritifera is unaffected by high pCO2 (Le Moullac et al., 2016). Likewise, Wang et al. (2015) recorded positive SFG values at different combinations of temperature and pH in the mussel M. coruscus. In contrast, Xu et al. (2016) found that elevated pCO2 in the seawater resulted in lower SFG for the clam R. philippinarum. The presence of PST in the diet significantly affected the SFG of mussels (M. chilensis) from Huelmo Bay, which responded in a similar manner to individuals from other populations of this species (Navarro et al., 2014; Navarro and Contreras, 2010). The initial decrease in SFG of M. chilensis exposed to PST was attributable to a lower clearance rate, which is consistent with results for other filter feeders (Lassus et al., 2004; Li et al., 2002). The recovery in scope for growth after exposure confirms the resistance of M. chilensis to harmful algae blooms events, demonstrating its ability to incorporate A. catenella into its diet (Bricejel et al., 2005; Fernández-Reiriz et al., 2008). A significant effect of the factor time and/or of the interaction of time with the other fixed factors was found for most of the physiological variables measured (feeding, metabolism, scope for growth), which can be explained by the high individual variability described for mussels exposed to different environmental conditions.

These findings are important considering the ecological and commercial importance of M. chilensis in the austral zone of Chile, where today over 280,000 tons are produced annually in suspended culture, and where harmful algal blooms are highly frequent.

5. Conclusions

The association between pCO2 and paralytic shellfish toxin (PST) may result in an indirect effect on mussel fitness. In fact, this study showed an inhibition of the processes associated with the acquisition of energy in mussels exposed to a diet containing PST. Therefore, the indirect effect of the high levels of pCO2 combined with paralytic shellfish toxins (PST) on mussel physiology, limits our understanding to know if this population will be able to adapt to the worst atmospheric scenarios projected for the next century, and points out the need to investigate the indirect effect that CO2 would have on marine organisms.

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