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Short Communication

Electrophoretic protein profiles of mid-sized copepod *Calanoides patagoniensis* steadily fed bloom-forming diatoms

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ABSTRACT. Recent field and experimental evidence collected in the southern upwelling region off Concepción (36°5'S, 73°3'W) showed an abrupt reduction (<72 h) in the egg production rates (EPR) of copepods when they were fed steadily and solely with the local bloom-forming diatom *Thalassiosira rotula.* Because diatoms were biochemically similar to dinoflagellate *Prorocentrum minimum*, a diet which supported higher reproductive outcomes, the fecundity reduction observed in copepod females fed with the diatom may have obeyed to post-ingestive processes, giving rise to resources reallocation. This hypothesis was tested by comparing feeding (clearance and ingestion rates), reproduction (EPR and hatching success) and the structure of protein profiles (*i.e.*, number and intensity of electrophoretic bands) of copepods (adults and eggs) incubated during 96 h with the two food conditions. The structure of protein profiles included molecular sizes that were calculated from the relative mobility of protein standards against the logarithm of their molecular sizes. After assessing the experimental conditions, feeding decreased over time for those females fed with *T. rotula*, while reproduction was higher in females fed with *P. minimum*. Electrophoretic profiles resulted similar mostly at a banding region of 100 to 89-kDa, while they showed partial differences around the region of 56-kDa band, especially in those females fed and eggs produced with *T. rotula*. Due to reproductive volume was impacted while larvae viability, a physiological processes with specific and high nutritional requirements, was independent on food type; post-ingestive processes, such as expression of stress-related proteins deviating resources to metabolic processes others than reproduction, are discussed under framework of nutritional-toxic mechanisms mediating copepod-diatoms relationships in productive upwelling areas.

Keywords: diatoms, blooms, food, copepods, reproduction, protein profiles.

Perfiles electroforéticos de proteínas del copépodo de talla media *Calanoides patagoniensis* alimentado sostenidamente con diatomeas formadoras de florecimientos

RESUMEN. Evidencia experimental y de campo recolectada en la región austral de surgencia frente a Concepción (36°5'S, 73°3'W), mostró una abrupta (<72 h) reducción en la tasa de producción de huevos (EPR) de copépodos cuando fueron alimentados sostenida y exclusivamente con cepas locales de la diatomea formadora de florecimientos masivos *Thalassiosira rotula.* En vista que las diatomeas fueron bioquímicamente similares al dinoflagelado *Prorocentrum minimum*, dieta que permitió mejores resultados reproductivos, la reducción en la fecundidad en hembras de copépodos alimentadas con diatomea pudo obedecer a procesos post-ingestivos, dando lugar a una redistribución de recursos nutricionales. Se evaluó esta hipótesis mediante la comparación de la alimentación (tasas de aclaramiento y de ingestión), reproducción (TPH y eclosión de huevos) y estructura de perfiles de proteínas (*i.e.*, número e intensidad de bandas electroforéticas) de copépodos (adultos y huevos) incubados durante 96 h en ambas condiciones de alimento. La estructura de los perfiles de proteínas incluyó los tamaños moleculares obtenidos desde la movilidad relativa de los estándares de proteínas contra el logaritmo de su peso molecular. Luego de evaluar las condiciones experimentales, la alimentación de hembras alimentadas

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con *T. rotula* disminuyó en el tiempo, mientras que la reproducción fue mayor en hembras alimentadas con *P. minimum*. Los perfiles electroforéticos resultaron mayormente similares en la región de la banda de 100 a 89-kDa, mientras que estos mostraron diferencias parciales en la región de la banda de 56-57-kDa, especialmente en aquellas hembras alimentadas y huevos producidos con *T. rotula*. Dado que el volumen reproductivo fue impactado mientras que la viabilidad de las larvas (proceso fisiológico con específicos y altos requerimientos nutricionales), fue independiente del tipo de alimento; procesos post-ingestivos, tales como la expresión de proteínas de estrés desviando recursos hacia otros procesos metabólicos distintos de la reproducción, se discuten en el marco de los mecanismos nutricionales-tóxicos mediando las relaciones copépodos-diatomeas en sistemas productivos de surgencia.

Palabras clave: diatomeas, florecimientos masivos, alimento, copépodos, reproducción, perfiles de proteínas.

Inter-specific relationship between primary producers and their consumers in the ocean involve multiple and diverse mechanisms that from the trophodynamic viewpoint ultimately modulate how much photosynthetic carbon is available for higher trophic levels. A specific issue of this relationship concerns the "goodness" of food for marine grazers represented by diatom blooms, which are highly prevalent biological features in the most productive ocean ecosystems (Irigoien *et al.*, 2002). In terms of food for copepods, main diatom grazers, such conditions are determined by the size-spectra, cell concentration, and biochemical properties of species forming the blooms (Jones & Flynn, 2005; Flynn, 2008; Koski *et al.*, 2008). On the matter, diversity and nutritional value associated to these microalgae aggregations can be greatly decreased by allelophatic mechanisms during the establishment and prevalence of the bloom (Legrand *et al.*, 2001; Flynn, 2008).

Chemical interactions among algae during blooms may in turn modify diversity and prey size-structure available at the time for grazers (Legrand *et al.*, 2003). Since size-distribution of food particles may restrict the efficient detection and capture of prey by the copepods, diatom blooms may thus compromise the achievement of the food ration, especially for those mid and large-sized species with higher food requirements (Price & Paffenhöfer, 1984).

Functionally, the high cell concentrations observed during diatom blooms (Scholin *et al.*, 2000) may induce high ingestion rates and, hence, low gut passage time and incomplete digestion of the ingested cells (Besiktepe & Dam, 2002). Both, passage time and partial digestion modulate assimilation efficiency and growth of copepods (Dutz *et al.*, 2008). Ultimately, bloom forming diatoms as many others microalgae (Turner, 2014) are able to produce an array of biologically-active metabolites, many of which have been attributed as a form of grazing deterrent (Turner, 2014 and references therein). Thus, some chain-forming diatoms, such as the species *Thalassiosira rotula*, have been found capable to alternate from just physical to more complex and compensatory chemical defense mechanisms against grazers (Miralto *et al.*, 1999; Hamm *et al.*, 2003; Fontana *et al.*, 2007). Therefore, when copepods were fed with different strains of *T. rotula* their egg production dropped, their embryos failed to develop, or hatched into malformed nauplii that die soon after birth (Ianora & Miralto, 2010).

Calanoides patagoniensis (Copepoda, Calanoidea) is a mid-size copepod species (2.5-2.7 mm length) that co-exists with *T. rotula* in the productive southern upwelling regions of the Humboldt Current System, where this diatom is one of the most common and dominant phytoplankton species (Anabalón *et al.*, 2007; Vargas *et al.*, 2007). In these ecosystems, this diatoms species was associated with reproductive failures in other large-sized co-existing copepod species, *Calanus chilensis*, expressed as low egg production rates, low egg hatching, and high percentage of larvae abnormality (Poulet *et al.*, 2007).

More recently and studying reproductive traits of *C. patagoniensis* upon local *T. rotula* strains, winter flagellate assemblages, and *Prorocentrum minimum*; Aguilera & Escribano (2013) found that although of copepod egg viability was unaffected by food treatments, reproductive activity in the form of egg production rates resulted 30% lower after sustained (3 days) ingestion of *T. rotula* . Interestingly, both diets had similar and relatively low C:N ratios (*T. rotula*$_{\text{C:N ratio}}$ = 4.3; *P. minimum*$_{\text{C:N ratio}}$ = 3.3). That is to say, both diets provided relatively high nitrogen compounds and thus, metabolic process with high proteins demand, such as reproduction, should not be limited (Checkley, 1980). Whether tested diets were similar in providing C and N for copepod females, post-ingestive processes, such as the reorganization of nutritional compounds, could lead to changes in copepod egg production rates. We tested this possibility through the comparison of feeding and reproductive traits as well as electrophoresis gel profiles of copepod females steadily fed (96 h) with both food treatments and their spawned eggs.

Copepods were collected between spring of 2007 and summer of 2008 at the upper 20 m of a shallow nearshore station (*ca*. 5 km from the shoreline) in the upwelling area off Concepción, Chile, in southern Pacific Ocean (36°5'S, 73°3'W). Samples were collected through vertical hauls of a WP-2 net with a 200-µm mesh size, and equipped with a non-filtering 1 L cod-end. Immediately after sampling, the cod-end contents were transferred into a 60 L thermo box and transported to a laboratory at the Marine Biology Station of Dichato. Within 2 h of capture, fertilized and unda-maged females of *C. patagoniensis* were carefully sorted out using a dissecting microscope Leica Leitz MZ6. Mature and reproductive copepod females were selected and gently transferred into 0.2 µm filtered sea water using the following criteria: 1) fully integrated antenna, 2) presence and pigmentation of gonadal segment, 3) visual recognition of oocytes in vitellogenesis phase II (Yehezkel *et al.*, 2000). After sorting, females were acclimated by 24 h in filtered sea water without food before to starting the experiments (for more details please see Aguilera & Escribano, 2013). Food media to feed spring cohorts of copepods consisted in a *T. rotula* culture collected from the study area during the spring of 2007, when diatom blooms dominate the phytoplankton structure and biomass (Vargas *et al.*, 2006, 2007).

The most abundant diatom *T. rotula* was then successfully isolated and cultured into 0.2 µm filtered sea water enriched with K-medium at 12°C with a 12:12 light: dark cycle (Guillard & Ryther, 1962; Keller *et al.*, 1987). Additionnally, it was supplied a culture of the dinoflagellate *P. minimum* as food for copepod cohorts obtained during summer 2008: this alga has proved to be a suitable food resource that has widely been used on feeding and reproduction experiments with marine copepods (Paffenhöfer *et al.*, 2005). Both microalga cultures were supplied during their exponential growth phase to ensure their nutritional quality as food for copepods (Diekmann *et al.*, 2009). Linear dimensions of algae (length and width) were measured under the microscope to later determine volume and equivalent mean spherical diameter. Carbon and nitrogen content were measured in algae filtered onto precombusted filters using a Thermo Finningan EA FLASH 1112 elemental analyzer.

Four experimental series were performed with both food treatments, each one consisting on 96-h individual incubations with daily food renewing and daily monitoring of clearance (CR), ingestion (IR), egg production (EPR) and hatching success (H) in 30 mature copepod females. Animals for experiments were individually and gently pipetted into 300 mL acid-washed crystallizing dishes (300 mL glass capsules with concave walls and flat floor) and incubated in a temperature-controlled chamber (13 ± 1°C). The uses of dishes allow a better individual monitoring of simultaneous copepod responses, such egg production, and fecal pellets production. Whereas turbulent environment that eventually could impair fecal pellets is only subjected to the aquatic perturbations derived from copepod swimming, more dense eggs and fecal pellets are deposited in the flat floor or gently in the concave walls of experimental dishes without major impairments. Furthermore, *ad libitum* food supply (>100 µg C L^{-1}) based on fast growing cell supplied during their exponential growth phase should promote large-sized and dense pellets (Butler & Dam, 1994). Estimations of CR and IR, measured as cell removal, considered a food concentration of 194 ± 52 µg C L^{-1} (*T. rotula*) and 175 ± 41 µg C L^{-1} (*P. minimum*). Clearance or filtration rate is the volume of water cleared of food particles by a consumer per unit time, whereas IR is the amount of food particles ingested by the consumer per unit time (Båmstedt *et al.*, 2000). Six control dishes with no animals and six dishes containing single adult females were incubated by ~8 h and mixed periodically to avoid cell sedimentation in the case of diatoms. After sieving through 80 µm the content of experimental dishes (to separate eggs 151 ± 6 µm diameter, and fecal pellets >150 µm length), water volumes of all dishes were filtered directly onto 0.7 mm precombusted (450ºC) glass-fiber filters and then were analyzed for elemental compounds as above.

Thus, IR was expressed in carbon units (µg C f^{-1} d^{-1}) following standard method (Frost, 1972). Food media during reproductive experiments was daily renewed maintaining a similar food concentration as in feeding estimation experiments. In case of *T. rotula*, food media was periodically and gently mixed to minimize cells settling; in turn, eggs produced over 24 h by single females during the incubations were quantified to obtain daily averages of egg production rates (EPR). From these batches produced daily with both diets, random groups of 30 eggs were allowed to hatch after 60 h incubation in 3-5 mL of filtered sea water to estimate hatching success (H). The rest of the daily EPR was cleaned with filtered sea water and then were carefully concentrated into cryovials and kept at -80°C until electrophoretic analysis. When each 96 h experimental series ended, females were gently cumulated, cleaned and kept separated from egg samples at -80°C until electrophoretic analysis. Furthermore, a sample of copepod males collected throughout the study from field samples was also included to compare electrophoretic protein profile, due we did not control food intake by copepods in the field.

Total soluble proteins were extracted by mechanical disruption of samples (copepods and eggs) in 0.5 mL of extracting buffer (Tris 100 mM (pH 7.5), NaCl 100 mM, EDTA 5 mM, PMSF 1 mM) (Tartarotti & Torres, 2009). Samples were sonicated during 3 cycles of 10 s followed by 10 s rest in a vibracell sonics sonicator at 50% gain. Afterwards, the samples were centrifuged at 15000 g for 15 min at 4°C and the supernatant was recovered. The protein concentration was determined using the Bradford method (Bradford, 1976) and Biorad reagents according to the manufacturer instructions. Bovine serum albumin was utilized as standard. Approximately 10 µg of proteins were mixed with the appropriate volume of 4X Laemmli sample buffer, heated, and charged into a 12% SDS-polyacrylamide gel. The electrophoresis was run at 100 mA until the tracking dye reached the gel bottom. The gel was stained with Comassie blue in a mixture Ethanol, water, acetic acid in the proportion of 4:6:1. The gels were distained in the same mixture without the colorant. The molecular sizes were calculated from a calibration curve constructed from the relative mobility of the proteins standard against the logarithm of their molecular sizes.

The effect of food offer (*T. rotula* and *P. minimum*) and incubation length (h) was assessed on daily averages of CR, IR, EPR, and H through a two-way ANOVA test. Mean averages included into the analysis were computed by compiling daily averages observed during the four experimental series performed with each food treatment. The potential association between food C and N contents and copepod responses (IR, EPR, and H), as well as between IR and reproduction (EPR and H), was addressed by means of simple regression and Spearman correlation tests depending on the degree of deviation from normality. Due to some eggs accounted to determine daily average of EPR were destined later to estimate H, the eggs quantity finally available to develop electrophoretic analysis was rounded around 300 eggs. Therefore, the reported concentration of soluble proteins to elaborate protein profiles with females and eggs was expressed in terms of µg f^{-1} L^{-1} and µg egg^{-1} L^{-1}, respectively. Statistical analyses were performed using the software STAT version 7.0.

Feeding activity in terms of CR ranged between 32-40 (*T. rotula*) and between 30-45 mL f^{-1} d^{-1} (*P. minimum*), while IR fluctuated between 10-14 (*T. rotula*) and 7-11 µg C f^{-1} d^{-1} (*P. minimum*). These variations in copepod feeding responses are shown in Figs. 1a-1b, while their statistical comparisons appear in Table 1. After assessing the two feeding conditions, CR and IR decreased over time for those females fed with *T. rotula*, while CR and IR increased for those fed with *P. minimum*. For reproductive traits the analysis revealed that EPR (egg f^{-1} d^{-1}) ranged between 27 ± 6 (*T. rotula*) and 31 ± 4 (*P. minimum*), which tended to decreased over time for those females fed with *T. rotula*. Although EPR decreased after 48 h with *P. minimum*, it recovered to their original levels after 72 h, and remained high until the end of the experiments (Fig. 1c). The interaction between incubation length and food type resulted in smaller brood sizes that decreased fecundity about 40% in those females fed with *T. rotula*, after 72 h of incubation. Other hand, H was relatively high (>90%) with both food treatments (Fig. 1d), although H was statistically lower with *T. rotula* (92 ± 4%). Spearman correlation analysis of pooled elemental composition data of food types showed significant but antagonistic correlations between the N and C:N ratio of diet and copepod IR, and while the first one positive ($n = 16$, $R = 0.4$, P-value < 0.05) the latter was negative ($n = 16$, $R = -0.5$, P-value < 0.05). Likewise, EPR varied correlated and significantly with CR ($N = 16$, $R = 0.5$, P-value < 0.05).

Concentration of soluble proteins ranged from 2.72 to 6.29 µg f^{-1} L^{-1} in adults and from 0.26 to 0.42 µg egg^{-1} L^{-1} in eggs (Table 2). Between 6 and 10 electrophoretic bands were retained in SDS-polyacrylamide electrophoresis gel elaborated with females and egg preparations, respectively (Fig. 2). Proteins derived from female preparations have molecular weights varying between 56 and 219-kDa, whereas these ranged between 56 and 170-kDa in eggs-derived samples. In general terms female's electro-phoretic profiles fed both food treatments resulted quite similar although band at 56-kDa was more intense in those females fed with *T. rotula* (Fig. 2, S2), while the structure of electrophoretic profiles of eggs spawned by females fed *T. rotula* showed greater number of electrophoretic bands than eggs spawned by females fed *P. minimum*. These bands corresponded to proteins retained at 73 and 56-kDa at 56-kDa electrophoretic bands which were more intense in those eggs spawned by females fed with *T. rotula* (Fig. 2, S4).

This experimental exercise lies on the assumption that as mid-sized copepod, *C. patagoniensis* could face difficulties to diversify their diet under a massive diatoms bloom. In this sense, some authors have proposed that multi-algal consortiums would allow copepods to avoid poorly diverse food resources in the field; such that even during blooms, unicellular or short chains of individual diatom cells are dispersed, mixed and often consumed together with other taxa (Flynn & Irigoien, 2009). Moreover, as copepods have the ability to eat different food particles including a variety of planktonic groups in their daily ration, they may enhance the probability of obtaining a nutritionally com-

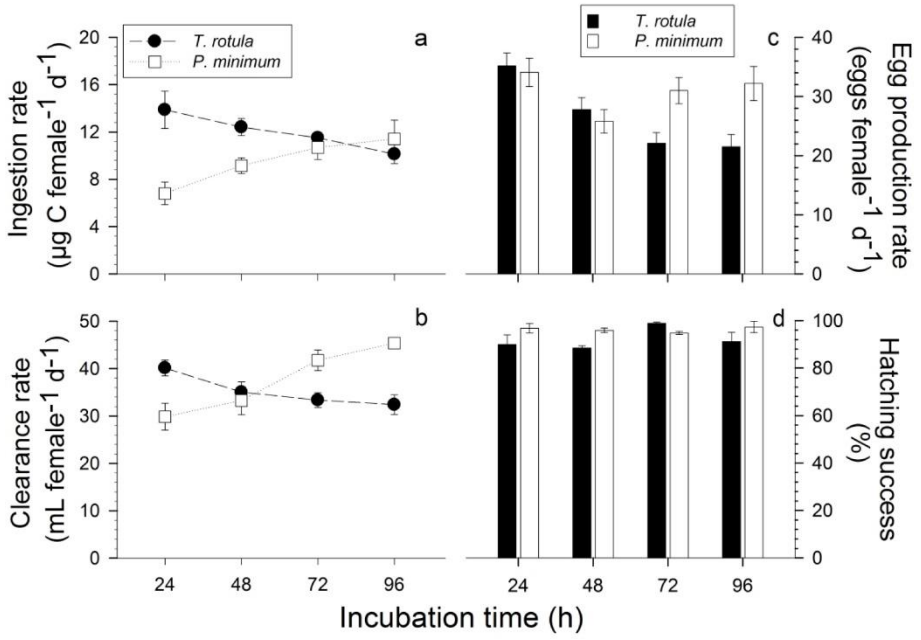

Figure 1. Simultaneous effect of food type (*Thalassiosira rotula* and *Prorocentrum minimum*) and feeding time on: a) ingestion rates, b) clearance rates, c) egg production rates, and d) hatching success of *Calanoides patagoniensis* during consecutive incubation experiments. Scatter plot as well as vertical bars denote daily means ± SD.

Table 1. Statistical results of two-way ANOVA analysis conducted to establish the effect of food treatment [*T. rotula* (T.r.) and *P. minimum* (P.m.)] and feeding time in copepod responses during consecutive incubation experiments. Copepod responses were: clearance rate (CR), ingestion rate (IR), egg production rate (EPR), and hatching success (H). Effect of incubation time is denoted as the trend that each response acquired over feeding time (equal, increase or decrease). df: degrees of freedom.

Variable	Factor	ANOVA	*F*-value	df	*P*-value
IR	Diet	P.m. < T.r.	34	1,16	0.0001
	Time	equal	0.5	3,16	> 0.05
	Interaction	-	18	3,16	0.0001
CR	Diet	P.m. > T.r.	7	1,16	0.02
	Time	increase	6	3,16	0.005
	Interaction	-	35	3,16	0.0001
EPR	Diet	P.m. > T.r.	21	1,16	0.0003
	Time	decrease	19	3,16	0.0001
	Interaction	-	13	3,16	0.0001
H	Diet	P.m. > T.r.	19	1,16	0.001
	Time	increase	4	3,16	0.02
	Interaction	-	8	3,16	0.001

plete ration in variable and nutritionally diluted environments (Kleppel, 1993). Certainly, this usually does occur in the ocean but elevated concentrations and spatial coverage of diatom blooms (Tiselius & Kuylenstierna, 1996; Miralto *et al.*, 2003; Vidoudez *et al.*, 2011) give them the character of mesoscale events that deserve special considerations. Firstly, diatom blooms are beyond a diluted environment in terms of food particles, and copepods tend to readily migrate and aggregate at localized diatom patches (Bainbridge, 1953; Tiselius, 1992; Atkinson & Shreeve, 1995; Bochdansky & Bollens, 2004). Further, bloom-forming diatoms are capable to reduce phytoplankton diversity through nutrients depletion, physical constraints, and allelophatic mechanisms (Price & Paffenhöfer, 1984; Legrand *et al.*, 2001; Turner, 2014). Thus, highly dense diatom blooms may induce a shortcut in the food diversity and field prey for mid and large-sized copepods, which could be more efficient feeding on large and highly abundant diatoms than small and diluted

Table 2. Details of electrophoretic banding (B) of produced with females and eggs of *C. patagoniensis* fed and produced after sustained feeding with *T. rotula* and *P. minimum*. This information primarily comprises protein complexes of high molecular weight, while molecular sizes of male bands were provided only as a reference. Sample size denotes number of females, eggs and males required for electrophoretic preparations.

Sample codes	Type of sample	Sample size	Food type (μg mL^{-1})	Total soluble proteins	Band codes (kDa)	Proteins molecular weights
S1	Females	72	*P. minimum*	195.66	B1-B2-B3-B4-B5-B6	213-183-150-133-113-62.9
S2	Females	65	*T. rotula*	295.75	B1-B2-B3-B4-B5-B6-B7	219-186-150-137-114-73-56
S3	Males	25	--	157.30	B1-B2-B3-B4-B5-B6-B7-B8-B9-B10	229-225-167-158-139-129-103-65-54-47
S4	Eggs	300	*P. minimum*	79.12	B1-B2-B3-B4-B5-B6-B7-B8	170-161-145-111-93-79-69-57
S5	Eggs	300	*T. rotula*	126.92	B1-B2-B3-B4-B5-B6	167-145-111-97-73-56

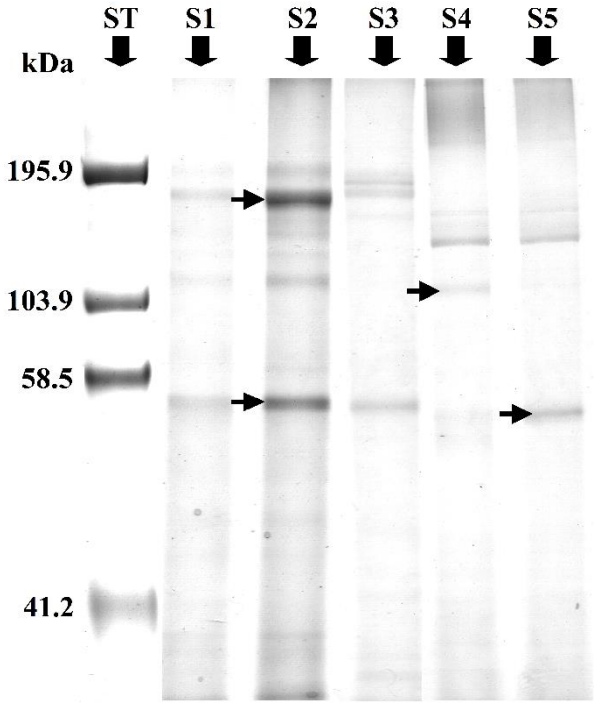

Figure 2. SDS-poly acrylamide gel electrophoresis profiles of copepods samples containing between 79 and up to 290 µg mL-1 of the total soluble proteins. Lanes: ST (protein standards with molecular sizes shown in kDa), S1 (females fed *P. minimum*), S2 (females fed *T. rotula*), S3 (males after-samplings preserved), S4 (eggs produced on *P. minimum*) and S5 (eggs produced on *T. rotula*). Black arrows highlight some specific electrophoretic bands: 167 and 56-kDa in S2, 113-kDa in S4, and 56-kDa in S5.

flagellates that possibly co-occur with the diatoms bloom.

We recently showed the egg production of *C. patagoniensis* steadily fed *T. rotula* decreased significantly after 72 h, besides these egg production rates were negatively associated with the IR and assimilation efficiency (AE) of *T. rotula* (Aguilera & Escribano, 2013); it suggests us sustained ingestion and assimilation of *T. rotula* could cause the drop of copepod gross growth efficiency (*i.e.,* carbon ingestion/egg mass production). Such kind of post-ingestive processes have been observed, for instance, under sustained stimulus of toxic compounds in the diet (Kozlowsky-Suzuki *et al.,* 2003); whereas other possible explanation considers the food quality that *T. rotula* represent for copepods. Current nutritional assessment was unfortunately limited since we only quantified and compared C and N contributions of both food treatments. This comparison revealed *T. rotula* reported the highest contribution of both elements (Aguilera & Escribano, 2013). Furthermore, previous feeding and reproductive studies developed in the study area indicate that diatoms (including *T. rotula*) were an adequate food resource to sustain secondary production (Vargas *et al.,* 2006) as well as reproductive performance of small-sized copepods (Aguilera *et al.,* 2011). Besides, both food treatments were supplied during their exponential growth phase to ensure their cellular goodness and thus, nutritional quality. Conversely to EPR, H (offspring viability) was relatively high (>90%) and unaffected by food type and incubation time, a reproductive outcome that has been previously documented in copepod females fed on several bloom-forming diatoms (Ianora & Miralto, 2010). Because larvae viability, a physiological process highly-demanding of specific nutritional resources, was not affected by the food treatments, it seems unlikely that a nutritional deficit may have caused the reproductive decline observed in those females fed on *T. rotula*.

Previous assumptions could be better understood by considering results of the comparison of protein profiles elaborated with females fed on- and eggs spawned with both food types. Protein profiles of copepod females resulted mostly similar in terms of

structure, although electrophoretic bands in the range of 60 till 200-kDa were more intensely expressed in females fed *T. rotula* (Fig. 2, S2). More dissimilar structures of protein profiles were observed in electrophoretic gels prepared with egg samples (Fig. 2, S4-S5). Thus, proteins in the retained in the band close to 103.9-kDa were only observed in egg spawned by females fed with *P. minimum*, while those in band of 56-kDa only observed in preparations derived from *T. rotula*. Several proteins with molecular weights of 86, 177, and 196-kDa circulate through the hemolymph and are transported to the growing oocytes during the second phase of crustacean vitellogenesis (Yehezkel *et al.,* 2000; Warrier & Subramoniam, 2002), providing a source of proteins, lipids, and carbohydrates to developing embryos (Wallace *et al.,* 1967; Adiyodi & Subramoniam, 1983; Shafir *et al.,* 1992). Due the electrophoretic bands that retained proteins in the band of 80-200 kDa were similar in profiles of females fed both food treatments, nutritional complexes such those mentioned above should have been available as demonstrated by the high and food-independent larvae viability.

Among microalgae species, the diatom *T. rotula* is considered capable of producing active metabolites with negative effects on his predators (Fontana *et al.,* 2007; Ribalet *et al.,* 2009, Caldwell, 2010). Through a specific metabolic pathway involving the oxidation of fatty acids, the local specie of *T. rotula* seems to be able to affect the physiology of the large-sized copepod *C. chilensis*, finally inducing their reproductive collapse (Poulet *et al.,* 2007). Such that, healthy females may experience reproductive impairments under sustained conditions of food containing or producing toxic compounds (Turner, 2014). We observed *C. patagoniensis* had a moderate AE on *T. rotula* (AE = 45%), inversely correlated with EPR and interpreted as AE was not entirely assigned to reproductive efforts (Aguilera & Escribano, 2013). Recent molecular evidence showed up- and down-regulation of stress-related proteins expression in *Calanus helgolandicus* after it was fed for 48 h on the oxylipin-producing diatom *Skeletonema marinoi* (Lauritano *et al.,* 2012). Regulation of gene expression was associated to the ability or inability to activate stress/detoxification proteins, such as the cytochrome P450 enzyme (CYP1A, 56-57-kDa) to cope with the toxic diet. In the current study we found that not only 56-kDa protein band was far more intense in copepods fed *T. rotula*, but it was also only present in eggs spawned by females fed diatoms. This may suggest that both stages could have activated stress/detoxification mechanisms to cope potentially detrimental compounds derived from eat diatoms. Interestingly, the majority of the eggs succeed to hatch despite the decline on egg production.

Chemical co-evolution between plant defenses and animal offenses has been proposed to explain some traits of the diatom-copepod relationship (Lauritano *et al.,* 2012); and both species, *T. rotula* and *C. patagoniensis*, co-exists and strongly interacts in this productive area during the upwelling period. The expression of non-essential proteins such that stress-related ones may represent new metabolic demands, that undermine other expensive processes like reproduction (Kurihara *et al.,* 2004), and growth (Chinnery & Williams, 2004). This possibility deserves to be further evaluated given the ecological and functional relevance of diatoms blooms in these highly productive marine ecosystems.

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