Chapter 3
Effects of Depuration on Subsequent Deterioration and Shelf Life of Cultured Grooved Carpet Shell Clam *Ruditapes decussatus* During Chilled Storage

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3.1 Introduction

The grooved carpet shell clam *Ruditapes decussatus* (L., 1758), which occurs from the eastern Atlantic to the Mediterranean, is one of the most consumed and profitable mollusks in the Mediterranean (Aníbal et al. 2011; FAO 2020). In Portugal, ca. 88% of the production is originated in the Ria Formosa (INE/DGRM 2019), a highly productive, 18.500 ha coastal lagoon system limited by a streak of barrier islands located in southern Portugal (36°58′N, 8°02′W to 37°03′N, 7°32′W) (Almeida and Soares 2012). *R. decussatus*' nutritional value, namely its high protein content, and very low level of fat, <1 g/100 g, and cholesterol (ca. 45 mg/100 g) make it a valuable seafood product. Condition and nutritional value (related to protein content) of clams in the Ria Formosa is higher in early-Summer (May–June) (Aníbal et al. 2011). Notwithstanding, like other filter-feeding species, there are risks associated with consumption derived from their contamination with chemicals (El-Shenawy 2004), microorganisms (Almeida and Soares 2012), or biotoxins

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(O'Mahony 2018; Vale et al. 2008). Presently, the classification system for the bivalves' production areas is based on bacteriological counts (*E. coli*) and heavy metals' contents (cadmium, lead and mercury) (EU 2004a, b, 2005a, 2006, 2007, 2008a, 2015; Ministério da Agricultura, do Desenvolvimento Rural e das Pescas 2006). Depending on the water quality of their environment, specimens have to be depurated, a remediation technique, before marketing (EU 2005a). Similar requirements and measures apply in the USA (US FDA 2009). Oliveira et al. (2011) provide an updated overview of this topic.

The process of depuration consists in maintaining the clams in cold and clean, sterile seawater for 24–48 h to eliminate or significantly reduce the bacterial load that was eventually accumulated during growth in production areas (Lee et al. 2008; Maffei et al. 2009; Ruano et al. 2012). Parameters such as shellfish suitability (in terms of salubrity and condition after harvesting and general handling), physiological conditions (*viz*. dissolved oxygen, loading, shellfish to water ratio, water flow, salinity, temperature, turbidity and disturbances), and infrastructures and operations (design of the operating system, basic hygiene draining, batch control, water quality) are critical (Lees et al. 2010). Clams are marketed live, packed in plastic net bags and stored at chill temperatures (ca. 5 °C) (Ministérios da Economia e da Inovação e da Agricultura, do Desenvolvimento Rural e das Pescas 2006). Their post-mortem spoilage dynamics is dependent on the concentration of substrates and metabolites, endogenous enzymes activity and microbial contamination (Sikorski and Kołakowski 2011) and is still also poorly understood (Anacleto et al. 2015). Commonly, parameters such as the survival of specimens, condition index (CI), glycogen content, pH, total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) contents, total viable counts (TVC), and/or abundances of *Escherichia coli*, coliforms, Enterobacteriaceae and psychrotrophic bacteria have been used to assess health status, spoilage dynamics and safety of bivalves subjected to distinct processing technologies and stored under diverse conditions. It is a common belief among clam farmers that depuration decreases appreciably the condition and quality of the individuals, lowering their marketability and economic revenue.

This study aimed to examine the effects of depuration on parameters of biological (mortality rate), physiological/commercial (condition index and percent edibility), physicochemical (pH and TVB-N content), microbiological (TVC, Enterobacteriaceae and psychrotrophic bacteria) and sensory quality of commercial-size clams stored at chill temperatures.

3.2 Material and Methods

3.2.1 Sampling

Samples of *R. decussatus* clams were obtained directly from a local licensed producer/certified depuration center. This ensured that all tested biological material was of commercial value and from the Ria Formosa, Algarve. Within 2–3 h after harvest or the agreed period of depuration (see below), the samples were transported to the laboratory in a refrigerated box and washed before experiments or analyses. At the start of each trial, a subset of clams was randomly selected for biometric measurements and for the determination of the percent edibility and condition index (see below).

3.2.2 Survival of Clams Kept in Refrigeration

The dynamics of survival of non-depurated clams were studied during the winter (December/January) and the late spring (May/June). Clams, n(winter) = 50 and n(summer) = 30, were kept under refrigerated conditions (5 ± 1 °C) for up to 30 days. Daily, specimens were individually and gently stimulated with a stiletto and the behavioral response noted as 1-fast valve reaction, 2-slow valve reaction; 3-no reaction but closed valves; and 4-no reaction and valves open. The number of specimens per day classified as 4 was used to estimate mortality rates and time required for 50% mortality (i.e. median time to death) (see below).

3.2.3 Effects of Storage Temperature on Post-Mortem Changes in pH and TVB-N Content

A sample (n = 194) of non-depurated clams were frozen at −18 °C beforehand to assure the post-mortem status to all specimens in the sample simultaneously. At the beginning of the trial, the clams were thawed at ambient temperature (ca. 20 °C) for 1 h and divided randomly into three groups. Each group was then maintained at 5, 15 and 25 °C. pH and TVB-N content were measured at regular intervals until the obvious deterioration of the samples in terms of sensory and chemical parameters.

3.2.4 *Effect of Depuration on Survival, Condition and Edibility, pH, TVB-N Content, Microbiological and Sensory Quality*

This experimental trial was conducted using n = 720 clams obtained in late Spring (during the season of peak consumption). Half of the specimens were depurated for 24 h in recirculated tanks filled with UV sterilized water at 14 °C at a licensed center in Olhão under customary commercial conditions employed in local facilities, while the other half was transported directly to laboratory in insulated box(es). In the lab, clams were stored packed in closed plastic net bags at chill temperatures (5 ± 1 °C) for up to 24 days.

At the start and at regular intervals, specimens were sampled to estimate the mortality (as in Sect. 3.2.2 above), condition index and percent edibility, pH, TVB-N content, microbiota abundance (TVC, Enterobacteriaceae and psychrotrophic bacteria counts) and sensory quality.

3.2.5 *Quality Parameters*

3.2.5.1 Condition Index and Percent Edibility

At the start of each experiment, individual clams were weighed (±0.1 mg) and their maximum length measured (to 0.05 mm) using a precision caliper. Clams were manually shucked by cutting the adductor muscle with a knife, and the meat was pressed with blotting paper to remove excess moisture before weighting (wet weight, WW, ±0.1 mg). The meat and shells were subsequently dried at 65 °C for 24 h and weighed again to obtain the dry weight (DW, ±0.1 mg). Condition index (CI) was calculated as

$$\mathrm{CI} = \left(\mathrm{MDW} / \mathrm{SDW}\right) \times 1000$$

where MDW is meat dry weight (g) and SDW is the shell dry weight (g) (Aníbal et al. 2011; Orban et al. 2011). Percent edibility (PE) was calculated as

$$\mathrm{PE} = \left(\mathrm{MWW} / \mathrm{TW}\right) \times 100\%$$

where MWW is meat wet weight (g), and TW is the total clam weight including the shell (g) (Aníbal et al. 2011; Mohite et al. 2008; Orban et al. 2011).

3.2.5.2 pH and TVB-N

The pH was measured directly in the meat of individual specimens using an appropriate probe connected to a pH meter (GLP21, Crison®, Spain). The TVB-N content was determined following the micro diffusion method of Conway as described in IPQ (2009) and instructed in the EU Regulation no. 2074/2005 (EU 2005b). Triplicate samples (10 g), each consisting of ca. 10 specimens were used for the assessment of TVB-N content.

3.2.5.3 Microbiological Parameters

Sample preparation was carried out following international standard practice (ISO 2003). In short, samples (20 g) of clams' meat were aseptically placed into sterile Stomacher® bags containing 90 ml of peptone water with NaCl (0.85% w/v) (Merck, Darmstadt, Germany) and homogenized for 1 min (Stomacher 400, Seward Ltd., London, UK). Aliquots of 1 ml were poured in Petri dishes according to serial decimal dilutions before the addition of appropriate media. For the enumeration of mesophilic aerobic (TVC) and psychrotrophic bacteria, plate count agar (PCA, Scharlau, Germany) was incubated at 30 °C for 72 ± 3 h (IPQ 2002) and at 6.5 °C for 10 days (ISO 2001), respectively. Enterobacteriaceae were enumerated after the inoculation of 1 ml aliquots into 10 ml of molten (at 45 °C) violet red bile glucose agar (VRBGA, Scharlau, Germany). After settling, a 10 ml overlay of molten media was added, and VRBGA plates were incubated at 37 °C for 24 ± 1 h (IPQ 1991). All plates were examined visually for typical colony types and morphological characteristics associated with each medium. Microbiological data, i.e. the number of colony-forming units (cfu) per unit mass were log-transformed prior to analysis, as log(cfu)/g.

3.2.5.4 Sensory Analysis

Sensory evaluation of raw and cooked clams was conducted on days 0, 1, 2, 3, 7, 11 and 14 during the storage at 5 °C using a panel of 11 individuals co-opted from the faculty, staff and graduate students of the Departamento de Engenharia Alimentar, Instituto Superior de Engenharia da Universidade do Algarve, that are experienced in the sensory assessment of seafood products.

Each panelist assessed seven sensory attributes of appearance, color (surface brightness, cream-ivory color, white/milky color and yellow/brownish color) and odor (intensity of fresh, ammoniac, and sulfide/putrid odors) of raw and cooked specimens using a 6-point scale (from 0, absent, to 5, extremely intense) that was adapted from Gonçalves et al. (2009) and Meilgaard et al. (2007). The panelists were also asked if at that time they would consume the samples or not. Raw clams were simply shucked before analysis whereas in the case of sensory analysis of

cooked clams, specimens were steamed in a microwave oven for 1 min (at 400 W) before being served to panelists.

3.2.6 Statistical Analysis

Results are given as mean ± standard deviation (descriptive statistics) or parameter estimates ± standard error (regression models). Comparisons of PE and CI among sampling dates in depurated and non-depurated clams were carried out using one-way ANOVA. Mortality (p) over storage time (t, in days) was modeled via logistic regression and the median time to death t_{50}, i.e. the time at which 50% of the specimens are dead, derived from the best fit model. The following form of the two-parameter logistic model was fitted,

$$p = \frac{1}{\left[1 + \exp^{\left(-r\left(t - t_{50}\right)\right)}\right]} \tag{3.1}$$

where p is the proportion of specimens alive, r is the rate (d^{-1}) and t_{50} is the median time to death (d). Dynamics of TVB-N content (y, mg N/100 g) over time (t, in days) was modeled using a first-order, exponential model,

$$y = a\exp\left(r\,t\right) + c \tag{3.2}$$

where a is the initial value, r is the rate (d^{-1}), and c is TVB-N content at t = 0. Clams' acceptability (measured as the proportion of total panelists willing to consume the clams at given day) was modeled using a modified version of the two-parameter logistic model common in psychometric research,

$$p = \frac{1}{\left[1 + \exp^{\left(-k\left(t - t_{50}\right)\right)}\right]} \tag{3.3}$$

that allowed the estimation of the median time to rejection, t_{50}. The exponential and the two-parameter logistic models were fitted via nonlinear regression analysis (using the Levenberg-Marquardt algorithm thru the function nls in R) and their goodness-of-fit assessed using the residual deviance and pseudo-R^2 (Ritz and Streibig 2009). All statistical procedures were carried out at the 0.05 level of significance and using R statistical software (R Core Team 2019).

3.3 Results and Discussion

3.3.1 Survival of Non-depurated Clams

The logistic model fitted the data on proportion alive over storage time well (pseudo-R^2 = 0.996 for Summer and pseudo-R^2 = 0.988 for Winter). The survival of non-depurated clams kept in refrigeration was substantially lower in Summer compared to Winter (Fig. 3.1). The median time to death, t_{50}, was 12.1 ± 0.05 d compared to 20.1 ± 0.22 d, respectively.

In a relatively similar experiment, Anacleto et al. (2013) kept non-depurated clams *Venerupis pullastra* and *Ruditapes philippinarum* at 4 °C and registered their mortality. These specimens' t_{50} were observed to be 5 and 14 days, respectively for *V. pullastra* and *R. philippinarum*. Marin et al. (2005) studied the effects of mechanical stress on under-sized *T. (Ruditapes) philippinarum* survival. Partly, the differences found in t_{50} among studies relate to methods of appraisal of clams' living. Moreover, the temperature difference between their environment/habitat and the refrigerating temperature is much more pronounced in Summer (commonly >20 °C vs. 5 ° C) than in Winter (~15 °C vs. 5 °C). Possibly, the greater thermal shock in Summer stresses animals considerably more such that survival is shortened.

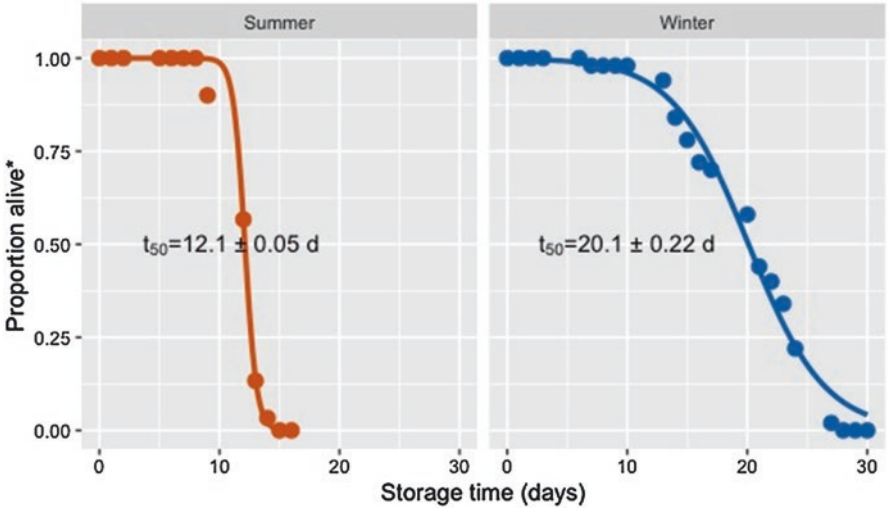

Fig. 3.1 Survival of non-depurated *R. decussatus* clams collected in Summer and Winter when in chilled storage. Proportion alive* (i.e. animals except those where shell gape occurred and external stimuli did not produce any response) and t_{50} is the median time to death (d) (n = 50 and 30 per season)

3.3.2 *Post-Mortem Changes*

The TVB-N content of non-depurated clams maintained at 5, 15 and 25 °C post-mortem (i.e. frozen at −18 °C before the experiment) were relatively low at the start of the trial (3.45 to 5.54 mg N/100 g) and increased exponentially with storage time, but at different rates (Fig. 3.2a). The rates increase from 0.31 ± 0.06 d^{-1} at 5 °C, to 0.72 ± 0.17 d^{-1} at 15 °C to 1.60 ± 0.22 d^{-1} at 25 °C. Considering the limits stipulated in the EU (EU 2005b, 2008b) (albeit for fish), the TVB-N content is estimated to exceed 35 mg N/100 g after 7, 2 and only 1 d, respectively for 5, 15 and 25 °C storage temperatures.

TVB-N concentrations in fresh fish are expected to be non-zero since ammonia is a metabolite already present (Pereira and Tenuta-Filho 2005). The same is anticipated for other seafood. Initial values found herein are in line with values reported by Cao et al. (2009) for Pacific oysters (*Crassostrea gigas*), 4.25 mg N/100 g, but

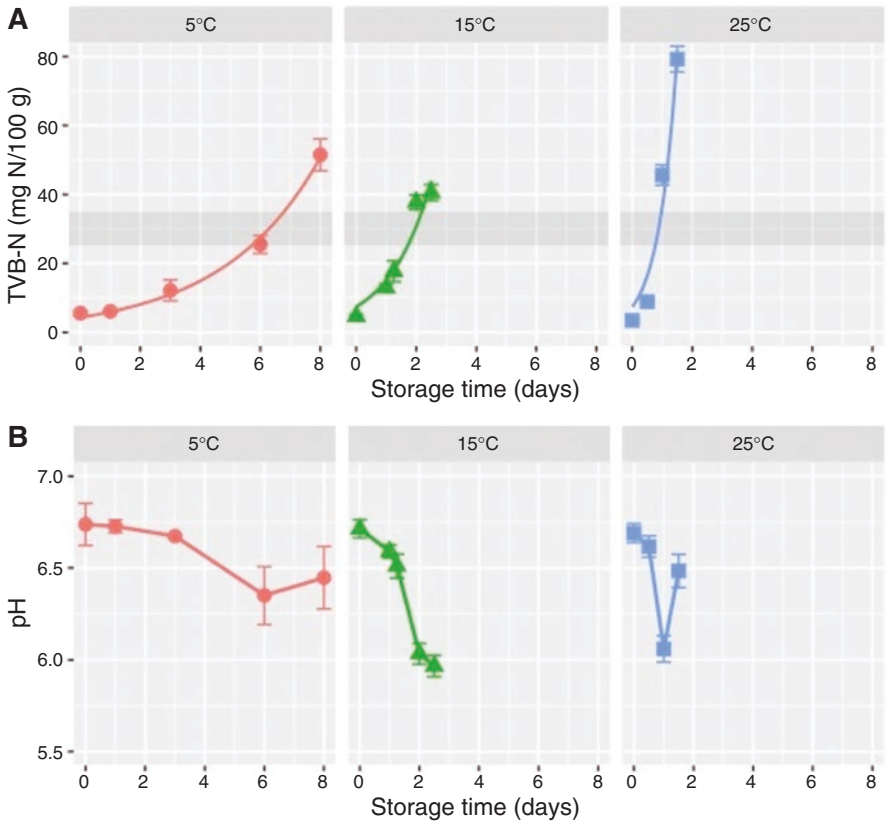

Fig. 3.2 Post-mortem changes in (**a**) TVB-N content and (**b**) pH in non-depurated, natural clams *R. decussatus* during storage at three distinct temperatures, 5, 15 and 25 °C (mean ± SD, n = 150 per treatment)

are substantially lower those reported by Rey et al. (2012) for clams (*V. rhomboideus*), 24 mg N/100 g, and for oyster (*Ostrea edulis*), 12–13.6 mg N/100 g, and by Tosun et al. (2018), Rey et al. (2012), Caglak et al. (2008), and Manousaridis et al. (2005) for raw mussels *Mytilus galloprovincialis*, 17.52 mg N/100 g, 18.99 mg N/100 g, 11.48 mg N/100 g and ca. 10 mg N/100 g, respectively. Values observed at the start of our trial are indicative of clams' freshness. Despite the low levels of non-protein nitrogenous compounds in bivalves, the production of volatile compounds resulting from the post-mortem degradation usually determines the increase in TVB-N content. Kim et al. (2002) recorded TVB-N values between 19 and 35 mg N/100 g for oysters packaged in LDPE pouches after 12 days of refrigerated storage. Manousaridis et al. (2005) reported increasing values of TVB-N content for control (vacuum-packaged) samples of shucked mussels up to 31.9 mg N/100 g after 12 days of storage at 5 °C. Similarly, Caglak et al. (2008) found rapidly increasing concentrations of TVB-N in the air- and vacuum-packaged shucked mussels up to 64 mg N/100 g (after 8 d) and 66.6 mg N/100 g (after 12 d). Herein, post-mortem concentration of TVB-N in clams stored refrigerated, at 5 °C, showed similar trends.

Although in pasteurized samples of marinated mussels *M. galloprovianclis* stored for 21 d at 4 °C, Tosun et al. (2018) observed increasing concentrations of TVB-N from 7.90 mg N/100 g after pasteurization to 29.24 mg N/100 g at the end of their experiment (21 days). After 12 d refrigerated storage, Caglak et al. (2008) registered TVB-N contents of 36.2–67.3 mg N/100 g in modified-atmosphere packaged shucked mussels.

Regarding pH (Fig. 3.2B), values decreased, sharply in clams kept at 15 °C and 25 °C, from initial values ranging from 6.69–6.71 to minima of 6.35 ± 0.16, 5.97 ± 0.06 and 6.06 ± 0.07 on days 6, 2.5 and 1, respectively at 5, 15 and 25 °C. Then, the pH values of clams stored at 25 °C increased steeply.

Herein pH values observed at the start of the storage trials were higher compared to those found by Erkan (2005) for shucked mussel, 5.96, by Cao et al. (2009) for Pacific oyster, 6.30, or by Manousaridis et al. (2005) for mussels, 6.3, but in line with the results reported in Caglak et al. (2008) also for mussel, 6.69–6.72. The initial reduction in pH values commonly occurs is seafood as a result of acid lactic formation ensuing autolytic processes, eventually from the conversion of glycogen to lactic acid as suggested by Cao et al. (2009); while the later increase in pH results from the production of basic compounds from nitrogen deamination (also reflected in TVB-N levels' increase), eventually due to microorganisms' metabolism (Huss 1995). Coincidently, the minima in pH values matched TVB-N levels reaching the concentrations legislated for fish (25–35 mg N/100 g) (see above) or proposed for bivalves (15–25 mg N/100 g; cf. Tosun et al. (2018)). This might suggest unacceptably low quality for Human consumption at those occasions. However, previous studies (Caglak et al. 2008; Erkan 2005) have been unsuccessful at finding a correlation between the pH and the freshness of bivalves as proposed by Pottinger (1948) for oysters (pH = 6.2–5.9 "good", pH = 5.8 "off", pH = 5.7–5.5 "musty", pH = 5.2 and below "sour or putrid").

3.3.3 *Depurated vs. Non-depurated*

We posited that depuration would have an impact on parameters of biological (mortality rate), physiological/commercial (condition index and percent edibility), physicochemical (pH and TVB-N content), microbiological (TVC, Enterobacteriaceae and psychrotrophic bacteria) and sensory quality of commercial-size clams stored at chill temperatures.

Results show (Fig. 3.3) that clams collected in late-Spring, during the period of peak consumption, subjected to depuration exhibit a similar survival trend to non-depurated specimens when kept refrigerated. The logistic model fitted well the data on proportion alive over storage time (pseudo-$R^2 = 0.979$ for depurated and pseudo-$R^2 = 0.996$ for non-depurated clams). The median time to death, t_{50}, practically overlapped, 13.0 ± 0.12 d vs. 12.1 ± 0.06 d respectively in depurated and non-depurated clams.

When comparing the survival rate of native *V. pullastra* and exotic *R. philippinarum* clams in the Tagus Estuary, Anacleto et al. (2013) found that t_{50} of depurated and non-depurated clams maintained at 4 °C were similar, 5 d for *V. pullastra* and 14 d for *R. philippinarum*. Those authors also experimented with higher storage temperature, 22 °C, to find that t_{50} were much shorter, 3 and 4 d. The median time to death, t_{50}, of 5 d was observed by Marin et al. (2005) that experimented with *T. philippinarum* at 18 °C. In another relatively similar experiment with depurated *T. (Ruditapes) decussatus* carried out at three different storage temperatures by Sadok et al. (2003), authors found that t_{50} ranged from 6.5 d for 5 °C, to 16 d for 10 °C, to 5 d for 20 °C. Bernárdez and Pastoriza (2011) observed increasing mortalities of mussels packed in 21% O_2 atmosphere (~atmospheric air) of up to ca.

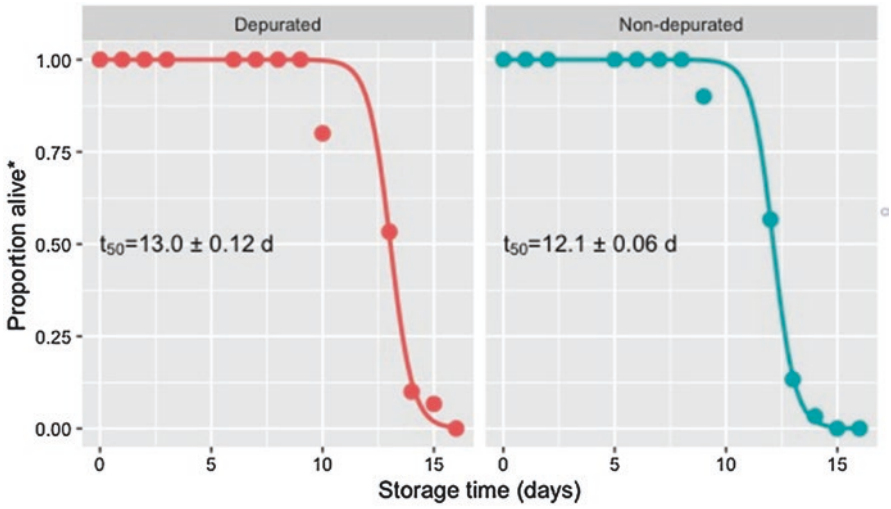

Fig. 3.3 Survival of depurated vs non-depurated clams *R. decussatus* collected in Summer and kept in chill storage at 5 ± 1 °C (n = 50 per treatment)

40% at the end of the 14-d storage period. Our results (t_{50} = 13 d) are close to the t_{50} reported by Anacleto et al. (2013) for 4 °C storage temperature (14 d) and the estimate of 16 days by Sadok et al. (2003) for the 10 °C storage temperature for *R. decussatus* clams. Notwithstanding, herein clams' mortality remained steadily low for up to 9 d, demonstrating clams' resistance to emersion, and corroborating that commercialization of clams in closed net bags outside water is the correct procedure.

In depurated clams, the condition index (CI) decreased and the percentage edibility (PE) increased during storage whereas in non-depurated clams CI remained constant and PE increased, the latter following a similar pattern to that observed for depurated clams (Fig. 3.4). Changes in CI and PE among times of storage were statistically significant (ANOVA, F = 4.69 with p = 0.0013 and F = 6.05 with p = 0.0002, respectively) in the case of depurated clams only. Notwithstanding, the variability of data cautions clear-cut conclusions.

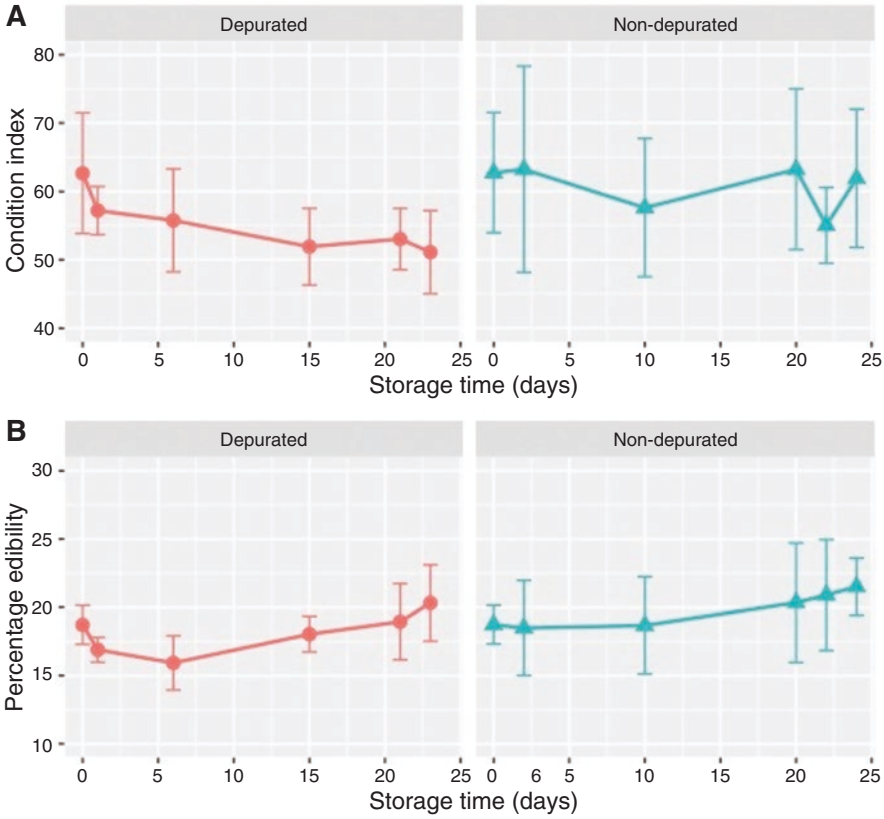

Fig. 3.4 Changes in (**a**) condition index (CI) and (**b**) percentage edibility (PE) of depurated vs. non-depurated clams *R. decussatus* (mean ± SD, n = 60 per treatment)

CI has been used as an ecophysiological measure of bivalve health status (Mubiana et al. 2006) and adopted in international trade as a standard criterion to select the best product (Aníbal et al. 2011). It may be considered as an indicator of fatness and marketability of commercially exploited bivalves (Orban et al. 2004). In this study, the initial CI and PE of clams were relatively lower than those reported by Aníbal et al. (2011) for the species in the Ria Formosa during late-Spring but reflect changing environmental conditions and physiological demands, the latter are assuredly dependent on the seasonality of the species' life/reproductive cycle (Ojea et al. 2004). Anacleto et al. (2013) also found decreasing trends in CI of clams during storage which they attributed to stressful conditions, namely the lack of feed that demands the use of biochemical reserves that decrease body weight (Albentosa et al. 2007). Seemingly, the depuration of clams in this study exacerbated those effects. In contrast, Gonçalves et al. (2009) observed non-significant changes in CI and PE of purified *R. decussatus* clams but in shorter 6-days storage trial (both in air and MAP).

Moreover, TVB-N content of depurated and non-depurated (live) clams stored chilled (+5 °C) exhibited similar exponential patterns (Fig. 3.5A). Exponential models fitted data quite well (pseudo-R^2 = 0.967 in depurated clams and pseudo-R^2 = 0.999 in non-depurated clams). Similar rates of (exponential) increase were found in depurated and non-depurated clams, 0.288 ± 0.058 d^{-1} and 0.256 ± 0.061 d^{-1} respectively. These were slightly lower than the rates estimated previously for post-mortem changes in TVB-N (see above) but higher than the rates reported by Sadok et al. (2003) for *T. decussatus* maintained at 5 °C, 0.104 d^{-1}. In their study, clams' TVB-N content reached ca. 10 mg N/100 after 10 d. Herein, a steep increase in TVB-N levels was observed after days 15–20. Seemingly, clams can sequester ammonia in the hemolymph (Ali and Nakamura 2000) for a few days after emersion, mitigating its toxicity. Afterward, the altered metabolism induced by emersion leads to the production of ammonia and other volatile bases from amines and amino acids as suggested by Sadok et al. (2003). Rey et al. (2012) observed an increase in TVB-N levels of depurated clams (*V. rhomboideus*), oysters (*O. edulis*) and mussels (*M. galloprovincialis*) stored under ozonized slurry ice or flake ice, especially in mussels, during their 6-days storage trial. The TVB-N contents exceeded the 25–35 mg N/100 g thresholds (stipulated for a number of fish species in Regulations (CE) no. 2074/2005 and no. 1022/2008) by day 20–21. If the proposed limits for bivalves (15–25 mg N/100 g; cf. Tosun et al. 2018) are used, then shorter times are estimated 17–18 days.

The pH decreased in the first 1–2 days, markedly in non-depurated clams (from 6.30 to 5.24); then, values increased to pH > 7 (Fig. 3.5B). This increase was more pronounced in non-depurated clams. Again, the initial reduction in pH values commonly occurs is seafood as a result of acid lactic formation ensuing autolytic processes, seemingly more pronounced in non-depurated clams, eventually from the conversion of glycogen to lactic acid as suggested by Cao et al. (2009); while the later increase in pH results from the production of basic compounds from nitrogen (also reflected in TVB-N levels changes). Gonçalves et al. (2009) reported

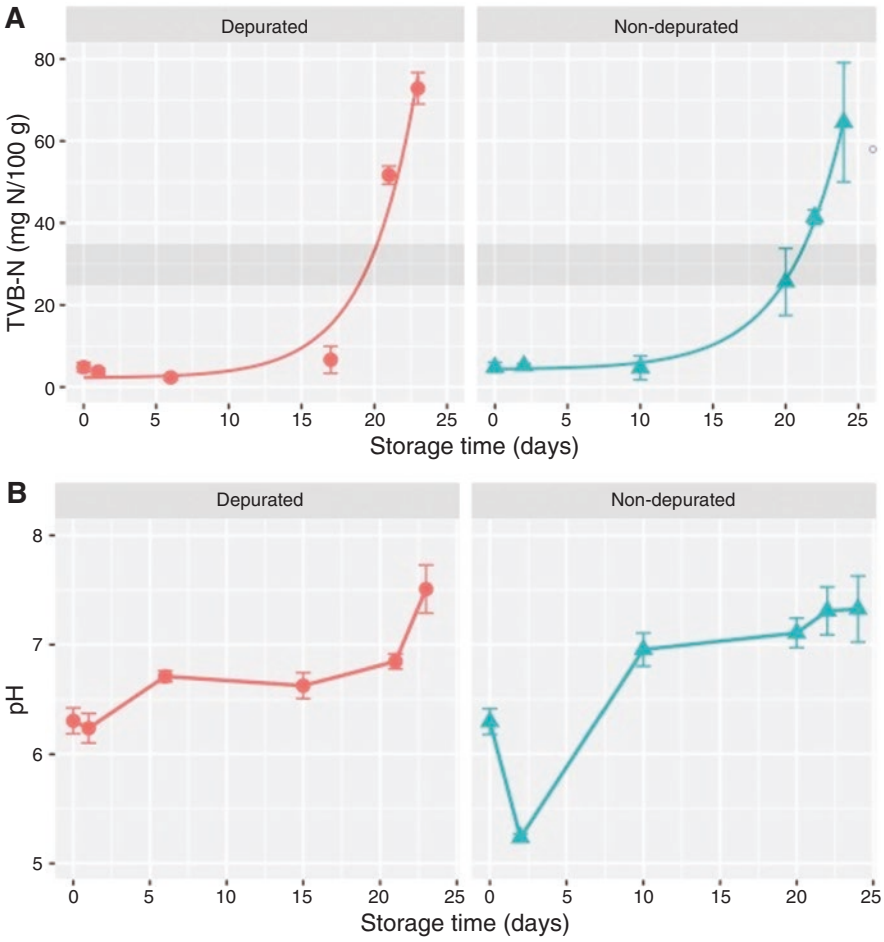

Fig. 3.5 Changes in (**a**) TVB-N content and (**b**) pH in depurated vs. non-depurated clams *R. decussatus* (mean ± SD, n = 180 per treatment)

increasing pH values in purified (commercially depurated) *R. decussatus* clams during a 6-day storage trial, they attributed to low glycogen utilization.

At the start of the trials, TVC averaged 4 log(cfu/g), and then decreased (Fig. 3.6a). This was noticeable and prolonged in depurated clams, to 3.27 log(cfu/g) during the first 6 d; while in non-depurated clams, the reduction was relatively smaller and shorter, to 3.7 log(cfu/g) in <2 d. Afterward, TVC increased in both groups following a similar pattern, but at comparatively higher levels in non-depurated clams (ca. +1–1.5 log(cfu/g)). In the latter, TVC exceeded 5.7 log(cfu/g) just after 20 days of chilled storage and capped at 6.70 log(cfu/g) on day 24, while depurated clams' TVC reached only 4.7 log(cfu/g) on day 23 of the experiment. Regarding Enterobacteriaceae (Fig. 3.6b), abundances remained relatively low, at ca. 1–2 log(cfu/g), during the first 20 days of the trial and then increased steeply in

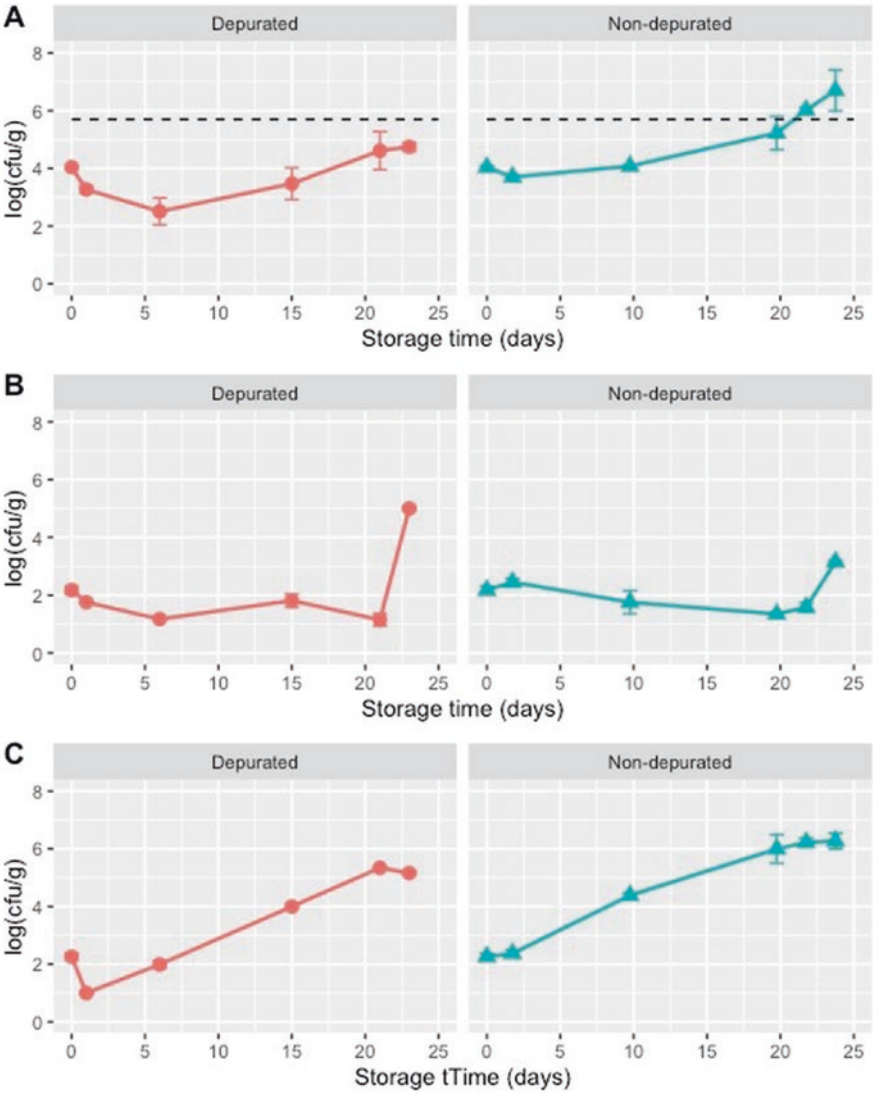

Fig. 3.6 (**a**) Total viable counts (TVC), (**b**) Enterobacteriaceae abundance and (**c**) psichrotrophic bacteria abundance (as log(cfu/g)) in (Summer) depurated vs. non-depurated clams *R. decussatus* (mean ± SD, n = 120 per treatment)

both depurated and non-depurated clams, to 5.0 log(cfu/g) and 3.2 log(cfu/g) respectively. The abundance of psychrotrophic bacteria (Fig. 3.6c) was reduced markedly after depuration, from 2.3 log(cfu/g) to 1.0 log(cfu/g), and then increased linearly to ca. 5 log(cfu/g); whereas in non-depurated clams, their abundance increased steadily from 2.3 log(cfu/g) on day 0 to >6 log(cfu/g) after day 20.

Expectedly, the depuration of clams had a noticeable effect on the microbiota assessed herein. Depuration efficiency is primarily related to size, siphoning activity, and physiological conditions of bivalves (Oliveira et al. 2011). However, other factors, such as the system design, water quality, oxygenation and flow rates, salinity, temperature, shellfish: water proportions, initial level of contamination, type and amount of pollutants, bivalve species and process duration are thought to influence depuration efficiency (Anacleto 2014). Moreover, depuration is highly effective in removing fecal bacterial from bivalves but less or even ineffective to remove other contaminants, e.g. virus, marine vibrios, toxins, or organic chemicals (Lee et al. 2008). El-shenawy (2004) observed reduced levels of microorganisms after the depuration of *R. decussatus* but only after 4 d. Martínez et al. (2009) reported significant reductions in pairwise aerobic plate count (APC) and psychrotrophic bacteria abundances in a set of farmed bivalves after depuration. In clams from the Tagus estuary, TVC were significantly reduced during depuration and later increased during "simulated transport" conditions at 4 °C and 22 °C (Anacleto et al. 2013). Increases of only ca. 1 log(cfu/g) were observed by Rey et al. (2012) in their 6-days storage trial of depurated clams, oysters and mussels using ozonized slurry ice and flake ice. The increase in TVC observed herein exceed the 5.7 log(cfu/g) limit recommended by ICMSF (1986) for bivalves or the 5 log(cfu/g) limit proposed by the National Advisory Committee on Microbiological Criteria for Foods (1992) only in the case of non-depurated clams and after 20 days of storage. Enterobacteriaceae, which include several pathogenic microorganisms, e.g. genus *Escherichia* (incl. *E. coli*), *Salmonella*, *Shigella* and *Yersinia*, may be present in the natural microbiota of foods or can be introduced as a result of post-process contamination (Baylis 2006) and pose food safety concerns since clams are filter feeders (Goulas and Kontominas 2007). Moreover, Enterobacteriaceae are known-causes of food spoilage, since their growth and metabolic activity results in off-flavors and odors and other organoleptic defects arising from the enzymatic breakdown of proteins and lipids (Baylis 2006). Herein, the abundances of Enterobacteriaceae remained low, 1–2 log(cfu/g), within the "borderline limit of acceptability" of 10^2–10^4 recommended in Forsythe (2000), for up to 20 d of storage. Psychrotrophic bacteria are the main contributors to the spoilage of seafood at refrigeration temperatures and thus can be used to estimate the microbial shelf life of seafood (Khan et al. 2005). Herein, only in clams subjected to depuration an initial reduction in the abundance of psychrotrophic bacteria was observed, otherwise, they grew almost linearly during the storage trial (Fig. 3.6c), taking advantage of their ability to grow at low temperatures (Bornert 2000). Considerable high abundances, >6 log(cfu/g), were found in non-depurated clams after 20 days. In contrast, Rey et al. (2012) were able to limit the growth of psychrotrophic bacteria in clams, oysters and mussels during storage for 6 days using ozonized slurry ice and flaked ice.

Concerning microbiological quality, i.e. TVC, Enterobacteriaceae and psychrotrophic bacteria abundances, "unacceptable" levels were reached long after usual consumption period of clams.

In terms of sensory acceptability, depurated and non-depurated raw clams (Fig. 3.7a) exhibited similar median times to rejection, t_{50}, 8.74 ± 0.56 d vs. 7.24 ± 0.56 d respectively. Moreover, on day 10, panelists characterized the clams' fresh, algae odor as very weak (scores of 1.2 vs. 4.2 on day 0) and noticed weak odors of ammonia and sulfides (scores of 1.54 and 1.3 vs. 0) in both depurated and non-depurated clams. In terms of appearance/color, clams were much less bright (scores of 2.8–2.9 vs. 4.3 on day 0) and lost their ivory hue (2.7–2.8 vs. 4.4), becoming milky (2.2–2.3 vs. 1.5) and yellowish (1.5–1.8 vs. 0.5). Similar findings for median time to rejection, t_{50}, were obtained when assessing cooked clams (Fig. 3.7b), 8.72 ± 0.62 d vs. 7.84 ± 0.62 d respectively for depurated and non-depurated clams. Changes in organoleptic attributes were also similar but less pronounced in terms of appearance/color.

In the study of Gonçalves et al. (2009) with live depurated *R. decussatus* clams stored for 6 days, panelists scored specimens at unacceptable levels on day 6, describing specimens' sensory attributes analogously to what was found herein, both in raw and in cooked samples. Moreover, Rey et al. (2012) found Spring-collected, depurated clams, oysters and mussels kept in ozonized slurry ice and flake ice to be acceptable for 4 days in terms of odor, appearance, taste and juiciness. Herein, both depurated and non-depurated clams were considered acceptable for relatively longer periods, plus 3–4 days.

3.4 Conclusion

The survival (t_{50}) of depurated and non-depurated clams stored chilled was similar, ca. 13 vs. 12 days, while their CI and PE changed relatively little (ca. 55–61% and 18–20% respectively). Also, during storage, pH decreased in the first 1–2 days, markedly in non-depurated clams (from ca. 6 to 5) and then gradually increased to values >7 on days 23–25. Concurrently, TVB-N increased exponentially in both depurated and non-depurated clams, exceeding EU limits by day 20. The initial microbial load was fairly low, 2 log cfu/g (Enterobacteriaceae and psychrotrophic bacteria) to 4 log cfu/g (TVC). Expectedly, after depuration microorganisms' abundance decreased, more pronouncedly (1–2 log cfu/g) in TVC and psychrotrophic bacteria. Afterward, abundances grew substantially to 5 log cfu/g at day 24. Enterobacteriaceae abundance remained constant till day 20 and then increased sharply. Similar dynamics of microorganisms' abundance were found for non-depurated clams but at comparatively higher abundances. In terms of sensory quality, the acceptability of depurated and non-depurated clams was similar to $t_{50} \approx$ 7–8 days, in both raw and cooked specimens.

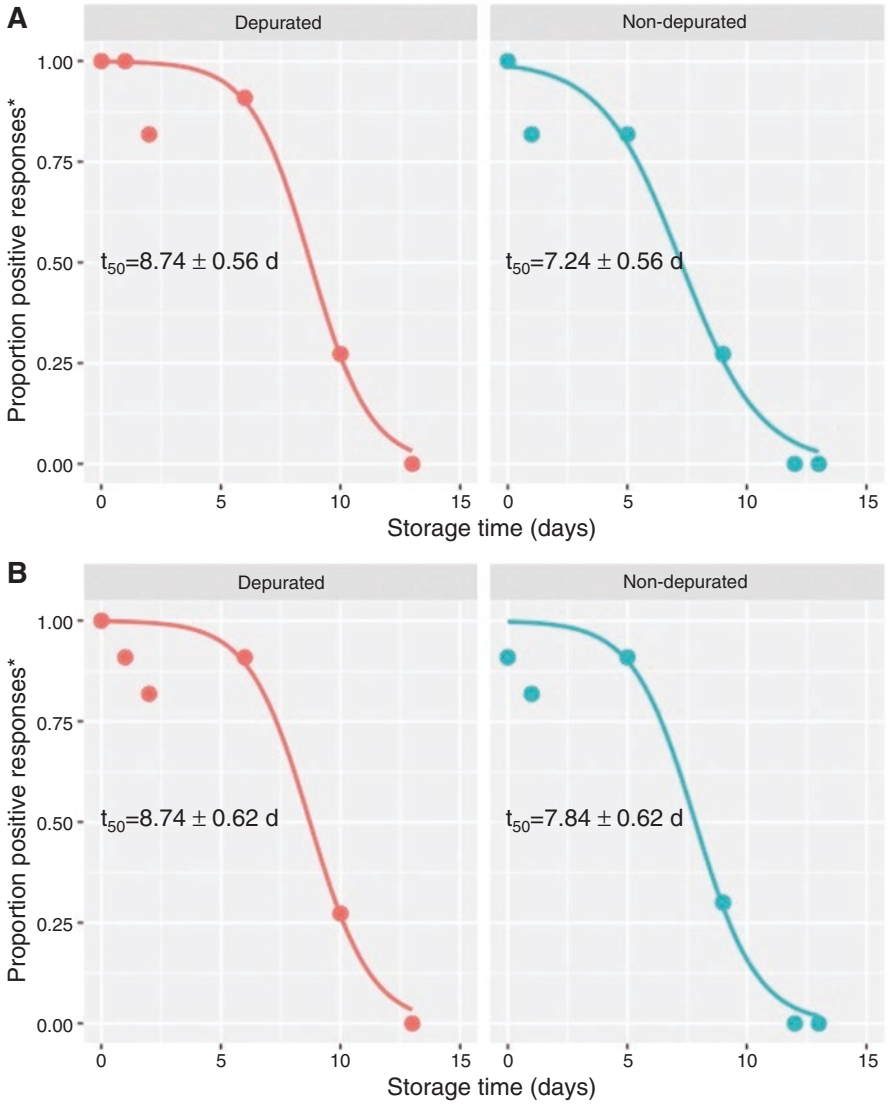

Fig. 3.7 Sensory acceptability of depurated vs. non-depurated (**a**) raw and (**b**) cooked clams *R. decussatus* (mean ± SD, n = 30 per treatment)

In sum, depuration affected in different ways the level but not the general dynamics of the quality parameters assessed during chilled storage of clams. However, eventual safety issues emerge long after habitual storage time and panelists' sensory rejection. Furthermore, our results indicate that the general belief among clam farmers that depuration decreases markedly the condition and quality of the specimens, lowering their marketability and economic revenue is not substantiated, reinforcing the importance of depurating bivalves as a means to ensure public health.

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References

Albentosa M, Fernández-Reiriz MJ, Labarta U, Pérez-camacho A (2007) Response of two species of clams, *Ruditapes decussatus* and *Venerupis pullastra*, to starvation: physiological and biochemical parameters. Comp Biochem Physiol B Biochem Mol Biol 146:241–249. https://doi.org/10.1016/j.cbpb.2006.10.109

Ali F, Nakamura K (2000) Metabolic characteristics of the Japanese clam *Ruditapes philippinarum* (Adams & Reeve) during aerial exposure. Aquac Res 31:157–165. https://doi.org/10.1046/j.1365-2109.2000.00402.x

Almeida C, Soares F (2012) Microbiological monitoring of bivalves from the Ria Formosa lagoon (south coast of Portugal): a 20 years of sanitary survey. Mar Pollut Bull 64:252–262. https://doi.org/10.1016/j.marpolbul.2011.11.025

Anacleto P (2014) Clams from Tagus estuary: microbiological, physiological and chemical responses to depuration, transport and environmental stress. Tese de Doutoramento, Faculdade de Ciências da Universidade de Lisboa, Lisboa

Anacleto P, Maulvault AL, Chaguri M, Pedro S, Nunes ML, Rosa R, Marques A (2013) Microbiological responses to depuration and transport of native and exotic clams at optimal and stressful temperatures. Food Microbiol 36:365–373. https://doi.org/10.1016/j.fm.2013.07.002

Anacleto P, Maulvault AL, Nunes ML, Carvalho ML, Rosa R, Marques A (2015) Effects of depuration on metal levels and health status of bivalve molluscs. Food Control 47:493–501. https://doi.org/10.1016/j.foodcont.2014.07.055

Aníbal J, Esteves E, Rocha C (2011) Seasonal variations in gross biochemical composition, percent edibility, and condition index of the clam Ruditapes decussatus cultivated in the Ria Formosa (South Portugal). J Shellfish Res 30:17–23. https://doi.org/10.2983/035.030.0104

Baylis CL (2006) Enterobacteriaceae. In: Blackburn C d W (ed) Food spoilage microorganisms. Woodhead Publishing, CRC Press, Cambridge, pp 624–667. https://doi.org/10.1533/9781845691417.5.624

Bernárdez M, Pastoriza L (2011) Quality of live packaged mussels during storage as a function of size and oxygen concentration. Food Control 22:257–265. https://doi.org/10.1016/j.foodcont.2010.07.007

Bornert G (2000) Importance des bactéries psychrotrophes en hygiène des denrées alimentaires. Rev Med Vet 151:1003–1010

Caglak E, Cakli S, Kilinc B (2008) Microbiological, chemical and sensory assessment of mussels (*Mytilus galloprovincialis*) stored under modified atmosphere packaging. Eur Food Res Technol 226:1293–1299. https://doi.org/10.1007/s00217-007-0657-1

Cao R, Xue C-H, Liu Q, Yong X (2009) Microbiological, chemical, and sensory assessment of pacific oysters (*Crassostrea gigas*) stored at different temperatures. Czech J Food Sci 27:102–108. https://doi.org/10.17221/166/2008-cjfs

El-Shenawy NS (2004) Heavy-metal and microbial depuration of the clam Ruditapes decussatus and its effect on bivalve behavior and physiology. Environ Toxicol 19:143–153. https://doi.org/10.1002/tox.20007

Erkan N (2005) Changes in quality characteristics during cold storage of shucked mussels (*Mytilus galloprovincialis*) and selected chemical decomposition indicators. J Sci Food Agric 85:2625–2630. https://doi.org/10.1002/jsfa.2331

EU (2004a) Regulation (EC) No. 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. Off J Eur Union L 139:55–205

EU (2004b) Regulation (EC) No. 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption. Off J Eur Union L 139:206–320

EU (2005a) Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Off J Eur Union L 322:1–26

EU (2005b) Commission Regulation (EC) No. 2074/2005 of 5 December 2005 laying down implementing measures for certain products under Reg. (EC) No. 853/2004 and for the organisation of official controls under Reg. (EC) No. 882/2004. Off J Eur Union

EU (2006) Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off J Eur Union L 364:5–24

EU (2007) Commission regulation (EC) No. 1441/2007 of 5 December 2007 amending Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs. Off J Eur Union L 322:12–29

EU (2008a) Commission Regulation (EC) No. 1021/2008 of 17 October 2008 amending Annexes I, II and III to Regulation (EC) No. 854/2004 and Regulation (EC) No. 2076/2005 as regards live bivalve molluscs, certain fishery products and staff assisting with official controls. Off J Eur Union L 277:15–17

EU (2008b) Commission Regulation (EC) No 1022/2008 of 17 October 2008 amending Regulation (EC) No 2074/2005 as regards the total volatile basic nitrogen (TVB-N) limits. Off J Eur Union L 277:18–20

EU (2015) Commission Regulation (EU) 2015/2285 of 8 December 2015 amending Annex II to Regulation (EC) No. 854/2004 as regards certain requirements for live bivalve molluscs, echinoderms, tunicates and marine gastropods and Annex I to Regulation (EC) No. 2073/2005. Off J Eur Union L 323:2–4

FAO (2020) Ruditapes decussatus [WWW Document]. http://www.fao.org/fishery/species/3542/en. Accessed 1 Mar 2020

Forsythe SJ (2000) The microbiology of safe food. Blackwell, Oxford. https://doi.org/10.1002/9780470999431

Gonçalves A, Pedro S, Duarte A, Nunes ML (2009) Effect of enriched oxygen atmosphere storage on the quality of live clams (*Ruditapes decussatus*). Int J Food Sci Technol 44:2598–2605. https://doi.org/10.1111/j.1365-2621.2009.02090.x

Goulas AE, Kontominas MG (2007) Combined effect of light salting, modified atmosphere packaging and oregano essential oil on the shelf-life of sea bream (*Sparus aurata*): biochemical and sensory attributes. Food Chem 100:287–296. https://doi.org/10.1016/j.foodchem.2005.09.045

Huss HH (1995) Quality and quality changes in fresh fish. FAO Fish Tech Pap 348:1–195

ICMSF (1986) Microorganisms in foods 2. Sampling for microbiological analysis: principles and specific applications. Blackwell, Oxford

INE/DGRM (2019) Estatisticas da Pesca 2018 [Fisheries statistics 2018]. Instituto Nacional de Estatística, Lisboa

IPQ (1991) NP 4137. Microbiologia alimentar. Regras gerais para a determinação de Enterobacteriaceae sem revitalização. Técnicas do número mais provável (NMP) e de contagem de colónias. Instituto Português da Qualidade, Lisboa

IPQ (2002) NP 4405. Microbiologia alimentar; Regras gerais para a contagem de microrganismos; Contagem de colónias a 30°C [Food microbiology; General guidance for the enumeration of micro-organisms; Colony count technique at 30°C]. Instituto Português da Qualidade, Lisboa

IPQ (2009) NP 2930. Produtos da pesca e da aquicultura. Determinação do teor de azoto básico volátil total (ABVT). Instituto Português da Qualidade, Lisboa

ISO (2001) ISO 17410. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of psychrotrophic microorganisms. International Organization for Standardization, Geneva

ISO (2003) ISO 6887-3: microbiology of the food chain — preparation of test samples, initial suspension and decimal dilutions for microbiological examination — part 3: specific rules for the preparation of fish and fishery products. International Organization for Standardization, Geneva

Khan MA, Parrish CC, Shahidi F (2005) Enumeration of total heterotrophic and psychrotrophic bacteria using different types of agar to evaluate the microbial quality of blue mussels (*Mytilus edulis*) and sea scallops (*Placopecten magellanicus*). Food Res Int 38:751–758. https://doi.org/10.1016/j.foodres.2005.03.001

Kim YM, Paik HD, Lee DS (2002) Shelf life characteristics of fresh oysters and ground beef as affected by bacteriocin-coated plastic packaging film. J Sci Food Agric 82:998–1003

Lee R, Lovatelli A, Ababouch L (2008) Bivalve depuration: fundamental and practice aspects, FAO. Fisheries technical paper. FAO, Rome

Lees D, Younger A, Doré B (2010) Depuration and relaying. In: Rees G, Pond K, Kay D, Bartram J, Santo Domingo J (eds) Safe management of shellfish and harvest waters. World Health Organization and IWA Publishing, London, pp 145–181

Maffei M, Vernocchi P, Lanciotti R, Guerzoni ME, Belletti N, Gardini F (2009) Depuration of striped venus clam (Chamelea gallina L.): effects on microorganisms, sand content, and mortality. J Food Sci 74:M1–M7. https://doi.org/10.1111/j.1750-3841.2008.00971.x

Manousaridis G, Nerantzaki A, Paleologos EK, Tsiotsias A, Savvaidis IN, Kontominas MG (2005) Effect of ozone on microbial, chemical and sensory attributes of shucked mussels. Food Microbiol 22:1–9. https://doi.org/10.1016/J.FM.2004.06.003

Marin MG, Moschino V, Meneghetti F, Da Ros L (2005) Effects of mechanical stress in undersized clams, *Tapes philippinarum*: a laboratory approach. Aquac Int 13:75–88. https://doi.org/10.1007/s10499-004-9029-z

Martínez O, Rodríguez-Calleja JM, Santos JA, Otero A, García-López ML (2009) Foodborne and indicator bacteria in farmed molluscan shellfish before and after depuration. J Food Prot 72:1443–1449. https://doi.org/10.4315/0362-028X-72.7.1443

Meilgaard MC, Carr BT, Civille GV, Carr BT, Civille GV, Carr BT (2007) Sensory evaluation techniques. CRC Press, Boca Raton. https://doi.org/10.1017/CBO9781107415324.004

Ministério da Agricultura, do Desenvolvimento Rural e das Pescas (2006) Decreto-Lei n.º 111/2006, transpõe para a ordem jurídica nacional a Directiva n.º 2004/41/CE. Diário da República 112/2006:4109–4112

Ministérios da Economia e da Inovação e da Agricultura, do Desenvolvimento Rural e das Pescas (2006) Portaria n.º 1421/2006, estabelece as regras de produção e comercialização de moluscos bivalves, equinodermes, tunicados e gastrópodes marinhos vivos. Diário da República 244/2006:8519–8520

Mohite SA, Mohite AS, Singh H (2008) On condition index and percentage edibiliy of the short-neck clam Paphia malabarica (Chemintz) from estuarine regions of Ratnagiri, west coast of India. Aquac Res 40:69–73. https://doi.org/10.1111/j.1365-2109.2008.02064.x

Mubiana VK, Vercauteren K, Blust R (2006) The influence of body size, condition index and tidal exposure on the variability in metal bioaccumulation in *Mytilus edulis*. Environ Pollut 144:272–279. https://doi.org/10.1016/j.envpol.2005.12.017

National Advisory Comittee on Microbiological Criteria for Foods (1992) Microbiological criteria for raw molluscan shellfish. J Food Prot 55:463–480. https://doi.org/10.4315/0362-028X-55.6.463

O'Mahony M (2018) EU regulatory risk management of marine biotoxins in the marine bivalve mollusc food-chain. Toxins 10:118. https://doi.org/10.3390/toxins10030118

Ojea J, Pazos AJ, Martínez D, Novoa S, Sánchez JL, Abad M (2004) Seasonal variation in weight and biochemical composition of the tissues of Ruditapes decussatus in relation to the gametogenic cycle. Aquaculture 238:451–468. https://doi.org/10.1016/j.aquaculture.2004.05.022

Oliveira J, Cunha A, Castilho F, Romalde JLL, Pereira MJJ (2011) Microbial contamination and purification of bivalve shellfish: crucial aspects in monitoring and future perspectives – a mini-review. Food Control 22:805–816. https://doi.org/10.1016/j.foodcont.2010.11.032

Orban E, Di Lena G, Masci M, Nevigato T, Casini I, Caproni R, Gambelli L, Pellizzato M (2004) Growth, nutritional quality and safety of oysters (*Crassostrea gigas*) cultured in the lagoon of Venice (Italy). J Sci Food Agric 84:1929–1938. https://doi.org/10.1002/jsfa.1896

Orban E, Di Lena G, Nevigato T, Masci M, Casini I, Caproni R (2011) Proximate, unsaponifiable lipid and fatty acid composition of bogue (*Boops boops*) and horse mackerel (*Trachurus trachurus*) from the Italian trawl fishery. J Food Compos Anal 24:1110–1116. https://doi.org/10.1016/j.jfca.2011.03.009

Pereira ÁAF, Tenuta-Filho A (2005) Avalialção de condições de consumo da sardinha Sardinella brasiliensis. Food Sci Technol 25:720–725

Pottinger SR (1948) Some data on pH and freshness of shucked eastern oysters. J Comm Fish Rev 10:9–11

R Core Team (2019) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Rey MS, Miranda JM, Aubourg SP, Barros-Velázquez J, Sanjuás-Rey M, Gallardo JM, Barros-Velázquez J, Aubourg SP, Rey MS, Miranda JM, Aubourg SP, Barros-Velázquez J (2012) Improved microbial and sensory quality of clams (Venerupis rhomboideus), oysters (*Ostrea edulis*) and mussels (*Mytilus galloprovincialis*) by refrigeration in a slurry ice packaging system. Int J Food Sci Technol 47:861–869. https://doi.org/10.1111/j.1365-2621.2011.02919.x

Ritz C, Streibig JC (2009) Nonlinear regression with R. Springer, New York. https://doi.org/10.1007/978-0-387-78171-6

Ruano F, Ramos P, Quaresma M, Bandarra NM, Pereira I (2012) Evolution of fatty acid profile and Condition Index in mollusc bivalves submitted to different depuration periods. Rev Port Cienc Vet 111:75–84

Sadok S, Uglow RF, Amor EA (2003) Nitrogenous compound changes in live, stored clam, tapes decussatus. J Aquat Food Prod Technol 12:113–128. https://doi.org/10.1300/j030v12n04_08

Sikorski ZE, Kołakowski E (2011) Seafood quality issues. In: Daczkowska-Kozon EG, Pan BS (eds) Environmental effects on seafood availability, safety, and quality. CRC Press, Boca Raton, pp 19–37

Tosun SY, Ucok Alakavuk D, Ulusoy S (2018) Quality changes of thermal pasteurized mussels (Mytilus galloprovincialis) during refrigerated storage at 4±1°C. Aquat Sci Eng 33:117–123. https://doi.org/10.26650/ase2018428669

US FDA (2009) National Shellfish Sanitation Program. Guide for the control of molluscan shellfish. (2007 Revision). US FDA, Washington DC

Vale P, Botelho MJ, Rodrigues SM, Gomes SS, Sampayo MADM (2008) Two decades of marine biotoxin monitoring in bivalves from Portugal (1986-2006): a review of exposure assessment. Harmful Algae 7:11–25. https://doi.org/10.1016/j.hal.2007.05.002