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AMMONIUM REMOVAL DURING LABORATORY CULTURE OF THE TEHUELCHE SCALLOP *AEQUIPECTEN TEHUELCHUS*

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ABSTRACT Laboratory-scale research involving marine bivalves maintained in recirculating or closed aquaria requires control of seawater quality parameters. Among them, the total dissolved ammonium nitrogen (TAN), NH_4^+ + ammonia (NH_3) nitrogen, concentration is a critical parameter because of its potential toxicity, mainly in closed systems. This study assessed the efficacy of two macroalgal species collected in Golfo Nuevo (Patagonia, Argentina) in removing TAN from the seawater of experimental systems containing the Tehuelche scallop (*Aequipecten tehuelchus*). The use of the traditional nitrifying bacterial biofilter was also explored. Scallops were collected from the Gulf of San José (Patagonia, Argentina) where they support an artisanal fishery of great socioeconomic importance. This resource is currently threatened by declining landings observed in the previous years, and experimental research is needed to explore the best conditions for *ex situ* cultivation. A 14 day-experiment was conducted in 3-L beakers containing an adult scallop with addition of a biofilter (treatment T1: containing *Ulva* spp.; T2: *Undaria pinnatifida*; T3: bacterial biofilter; and T4: mechanical filtration before *Ulva* spp. addition). Negative controls (NC) (no scallop or biofiltration) and positive controls (PC) (without biofiltration) were also performed. The concentration of TAN was measured at the beginning of the experiment and every 48 h, and temporal patterns were described by regression models. Removal efficiency relative to PC was calculated on days 8 (Rd8%) and 14 (Rd14%). Ammonia concentration was estimated as a function of seawater temperature, pH, and salinity. In PC, TAN increased at a rate of 0.03 $mg{\cdot}L^{-1}{\cdot}h^{-1}$, reaching a final mean value of 9.85 $mg{\cdot}L^{-1}$ for total dissolved NH_4^+-N + NH_3-N (TAN-N) (12.69 $mg{\cdot}L^{-1}$ TAN) and 0.15 $mg{\cdot}L^{-1}$ for NH_3. In T1, TAN ranged from 0.02 to 0.22 $mg{\cdot}L^{-1}$ TAN-N during the first 10 days of the experiment (below those measured in PC, T2, and T3), and Rd8 and Rd14 were 98% and 84%, respectively. The highest TAN concentrations observed in the whole experiment were measured in T2, exceeding those in PC. In T3, TAN-N mean concentrations on days 8 and 14 were 1.45 $mg{\cdot}L^{-1}$ (Rd8 73%) and 0.45 (Rd14 95%), respectively. In T4, a linear increase of TAN was observed during the mechanical filtration period; after *Ulva* spp. addition, TAN decreased to levels as low as those measured in NC (<0.025 $mg{\cdot}L^{-1}$ TAN-N) until the end of the experiment (Rd14 100%). These results show that Tehuelche scallops may be relatively resistant to TAN and that NH_3 concentrations were higher than several safety standards. The seaweed *Ulva* spp. provided the most efficient biofilter between both tested seaweeds, and its usage would represent a good alternative for the traditional bacterial biofilter in small-scale aquaculture experiments, including long-term acclimation. This information is useful for conducting experimental assays of this overexploited artisanal scallop resource from the Patagonian coast.

KEY WORDS: dissolved nitrogen, biofilters, seaweeds, *Aequipecten tehuelchus*, scallop, aquaculture

INTRODUCTION

Laboratory-scale research on the physiology, nutrition, toxicology, and reproduction of bivalves often requires prolonged maintenance of the targeted study organisms in relatively small and static aquarium systems with little or no water exchange. Therefore, the quality of seawater is one of the most important aspects to consider in such studies. Among toxic or potentially toxic compounds, metabolic by-products, such as ammonia (NH_3) and carbon dioxide (CO_2), are very likely to occur. The latter may not be particularly toxic, provided that sufficient dissolved oxygen (DO) is available, but CO_2 acidity may be enhanced. In general, the acidification of seawater in aquaria is a long-term process due to carbonate buffering, yet it could become problematic in some cases such as when fish are fed in ponds (Kikuchi et al. 1994). Bivalves, in particular, can be tolerant of CO_2 acidification (Fernández-Reiriz et al. 2012, Lee et al. 2016). Significant negative effects have been reported at very high CO_2 concentrations (~2,000–4,000 ppm), at a decreased pH of ~0.7, and after several days of exposure (in some cases after ~80 days) (Saphoerster 2008, Fernández-Reiriz et al. 2011, Fernández-Reiriz et al. 2012). In addition, Lee et al. (2016) documented sublethal effects when the pH was below 6.5 and reported a median lethal pH after 96-h exposure (LpH_{50} 96 h) of ~5.4. It must also be taken into account that the sensitivity of the organisms is dependent on body size, life history stage, location, and history of natural CO_2 exposure, among other factors (Saphoerster 2008, Range et al. 2014, Lee et al. 2016).

Total dissolved ammonium nitrogen (TAN) is an excretion product of aquatic animals which can become hazardous to organisms even over short time periods (Colt & Armstrong 1981, Lawson 1995, Losordo et al. 1999, Masser et al. 1999, Buzin et al. 2015). Dissolved inorganic nitrogen (TAN) is the sum of two chemical forms, ionized ammonium (NH_4^+-N) and deionized ammonium nitrogen or ammonia (NH_3-N), whose

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dynamic equilibrium depends mostly on pH, temperature, and salinity. The dissociation constant of NH_4^+ (pK) in seawater is equal to 9.5 at 25°C. At a pH of 8.1, 95% of TAN is present as NH_4^+ and 5% as NH_3 (Millero & Sohn 1992). In general, at the pH of seawater (7.9–8.2; Huguenin & Colt 1989) a high proportion of TAN is present as the less-toxic species (NH_4^+-N) whereas NH_3-N, the most toxic form, remains at low levels (Lawson 1995, Collos & Harrison 2014, Buzin et al. 2015).

When the TAN concentration is poorly controlled in research experiments, it may lead to artifacts and meaningless results. In general, acclimation of the experimental organisms is necessary before the assay starts, for a period that could range from a few days up to several weeks. Over the duration of the experiment, bivalves are often maintained at high densities in closed aquaria, increasing the risk of TAN buildup (Canesi et al. 2014, Chen et al. 2016, Kuranchie-Mensah et al. 2016, Guo et al. 2017). Experimental conditions, especially the food supply, will affect the concentration of TAN over time (Ansell & Sivadas 1973, Bayne & Scullard 1977, Merino et al. 2009, Soria et al. 2011). In the case of bivalves, the organisms can be kept under starvation conditions, as is the case in several studies of cellular growth and maintenance capacity (Husmann et al. 2016), depuration of phycotoxins (Svensson & Förlin 2004), other metabolic and toxicological studies (Ansell & Sivadas 1973, Xu et al. 2016), bacterial depuration (Pereira et al. 2017), and TAN-exposure experiments (Pagliarani et al. 2008).

In most cases, the control of TAN is performed through total or partial seawater exchange. This could create a stressful environment, however, and could thus be especially counterproductive if indicators of stress are under investigation (Fang et al. 2008, Thompson et al. 2012). Other TAN removal techniques have been developed for use in aquaculture, which could also be implemented in laboratory-scale aquaria. These include removal by nitrifying bacteria and seaweeds (Losordo et al. 1999). Traditional bacterial biofiltration has been widely studied and used, mostly in freshwater recirculation systems (Greiner & Timmons 1998, Losordo et al. 1999, Menasveta et al. 2001, Seo et al. 2001, Pedersen et al. 2015, von Ahnen et al. 2015, González-Silva et al. 2016, Huang et al. 2016). Bacteria, however, are very sensitive to temperature and nutrient changes, and to the presence of some chemicals, such as metal-binding compounds and amines, among others (Hooper & Terry 1973). Seaweeds provide an alternative to bacterial biofilters. They consume nutrients directly from the seawater [carbon (C), nitrogen (N), and phosphorus (P)] and their biomass can be easily removed from the system. Moreover, with the use of macroalgae, the physicochemical conditions of the system remain more stable, and checking oxygen, pH, and alkalinity levels is less frequently required (Schuenhoff et al. 2003, Neori et al. 2004).

At an experimental scale, the present study explores the dynamics and efficiency of TAN removal of two macroalgal species collected in Golfo Nuevo (Patagonia, Argentina), for comparison with the traditional bacterial biofilter. The bivalve selected for the study was the Tehuelche scallop [*Aequipecten tehuelchus* (d'Orbigny, 1842)] from the Gulf of San José (Patagonia, Argentina). Among the bivalves of economic importance in Argentina, some also with aquaculture potential (Zaixso 1980, Pascual & Zampatti 1998, Álvarez et al. 2012, Soria et al. 2016), *A. tehuelchus* supports an artisanal fishery in northern Patagonia (64.13° W 42.15° S–64.20° W 42.20° S) (Soria et al. 2016). Because landings have been declining in recent years, experimental research on this species, aimed at exploring the best conditions for grow out cultivation, is needed. Results of this study will thus contribute to the development of aquaculture of this scallop species in the Patagonian region of South America.

MATERIALS AND METHODS

Seaweeds as Biofilter

The seaweeds used were the green alga *Ulva* spp. and kelp species *Undaria pinnatifida* that both grow in intertidal rocky shores of Golfo Nuevo (Piriz et al. 2003, Casas et al. 2008, Teso et al. 2009, Rechimont 2011). They were collected from Punta Este (Golfo Nuevo). Precautions were taken during harvesting to ensure complete holdfast extraction and avoid seaweed damage. In the laboratory, the seaweeds were cleaned and washed with seawater and acclimated for 2 days in a 30-L aquarium filled with 1-μm-filtered, ultraviolet-treated (Biolight Technologies: 2,000 L h^{-1}, 40 W) seawater at a temperature of 12.5°C, 12:12-h light:dark photoperiod, mean photon flux density of 10.65 μM ms^{-1} (measured with a Puntual PAR Cavadevices sensor), salinity of 33 (measured with Arcano FG-211 Salinity/ATC 0–100), and DO > 90% saturation level (aeration provided by airstones and DO measured with a YSI model 58 DO meter).

Bacterial Biofilter

An upflow plastic bead design was used for the bacterial biofilter. The filter media containing active bacterial biomass were obtained from an activated recirculation system. The plastic beads were substituted with 30 pieces of corrugated pipe (length: 3.5 cm each; mean total surface area for bacterial growth: ~1.05 m^2). The filter medium remained half-submerged and was constantly recirculated throughout the experiment.

Collection of the Tehuelche Scallop

Adult Tehuelche scallops were collected by scuba diving from the San Román bed, Gulf of San José (Chubut, Argentina), in March 2015 at an ambient temperature of 12°C–13°C. The bivalves were transported to the laboratory in thermally insulated boxes containing ice. After being cleaned of epibionts, they were placed in a 300-L recirculating system under the same room and seawater conditions as those described for seaweed acclimation. They were kept in this system without feeding until the beginning of the experiment (≤3 days).

Experimental Setup

The laboratory experiment was conducted in 3-L plastic beakers, previously cleaned with 5% HCl, subsequently washed with running freshwater, and finally with distilled water. Aquarium accessories were disinfected in 10% chlorine for 1 day, and then washed generously with running freshwater and distilled water. All materials were dried before conducting the experiment. Seawater was filtered through Whatman GF/C glass fiber filters (nominal pore size: 1.2 μm). The beakers were filled and then covered with plastic wrap to prevent evaporation. Room and seawater conditions were the same as those for seaweed acclimation.

Six different assays (two replicate beakers each) were carried out over 14 days: negative controls (NC) and positive controls (PC), and four treatments (T*n*) (Fig. 1), namely

Negative control: beaker without scallop or filtration.
Positive control: beaker with scallop and without filtration.
T1: *Ulva* spp. as biofilter (44–47 g wet weight, WW).
T2: *Undaria pinnatifida* as biofilter (43–46 g WW).
T3: bacterial biofilter.
T4: beaker with scallop and mechanical filtration of water during the first 8 days and biological filtration thereafter (the most effective among those used in T1 to T3), consisting of the addition of 44–47 g WW of *Ulva* spp. into each system, was used during the remaining 6 days (days 9–14). Mechanical filtration was conducted every 48 h by siphoning off the bottom water, which was then passed through a commercial coffee fabric filter to remove settled material and then fed back to the beaker. On the 8th day, mechanical filtration was discontinued. One scallop per beaker was used for each treatment and PC. Scallop weight ranged from 61 to 68 g total body WW, corresponding to a shell height between 69 and 74 mm.

During the experiment, pH was measured using a pH meter (RS232). Seawater aliquots of 50 mL (in duplicate) were taken from each beaker every 48 h to measure TAN concentration. Samples were always taken in the morning to reduce differences related to the circadian rhythm of bivalve excretion (Langton et al. 1977, Ellner et al. 1996). The total volume extracted was ~12% of the initial volume, and the beakers were not refilled. The samples were analyzed for NH_4^+-N using the colorimetric method indophenol blue reaction (Strickland & Parsons 1972, Parsons et al. 1989) and they were read with a ultraviolet-vis spectrophotometer at 640 nm wavelength.

The TAN concentrations were expressed as mg L^{-1} TAN-N (total dissolved NH_4^+-N + NH_3-N). The relative error was always <10% between chemical replicates and <35% between assay replicates. The concentration of NH_3-N in TAN was obtained from data tabulated by Zepka Baumgarten et al. (1996), who reported the percentage of NH_3-N in a sample, for different temperatures, pH, and salinities.

The efficiency of TAN removal (% *R*) in each treatment (*n*) at time *t* was calculated as

$$\% Rt = ([\mathrm{PC}t] - [Tnt]) * 100/[\mathrm{PC}],$$

where (PC*t*) is the TAN concentration in the PC at time *t* and (*Tn*) is the TAN concentration in treatment *n* at time *t*.

Statistical Analysis

Different regression models were fitted to the results: linear regression, second- and third-order polynomials, and exponential function. The Akaike information criterion with a correction for finite sample sizes was used to find the best model fit (Burnham & Anderson 2002). When $\Delta i \geq 2$, the models were considered to provide different fit to the data. The Akaike weights (Wi) are shown as a parameter of selection. The R Core Team software (2015) was used for all statistical analysis.

RESULTS

Scallops were healthy during all the experiments, as indicated by active filtration and an extended mantle and tentacles. Little variation was recorded in pH values (range: 7.8–8.0); the salinity remained constant at 33.

The initial mean TAN-N concentration in seawater was 0.020 ± 0.005 mg L^{-1} for all beakers. The TAN removal efficiency varied among treatments and also between controls (Fig. 2). The main TAN patterns in controls and treatments are described as follows:

In the NC, the TAN-N concentration remained relatively constant and below 0.025 mg L^{-1} (Fig. 3). Concentrations in

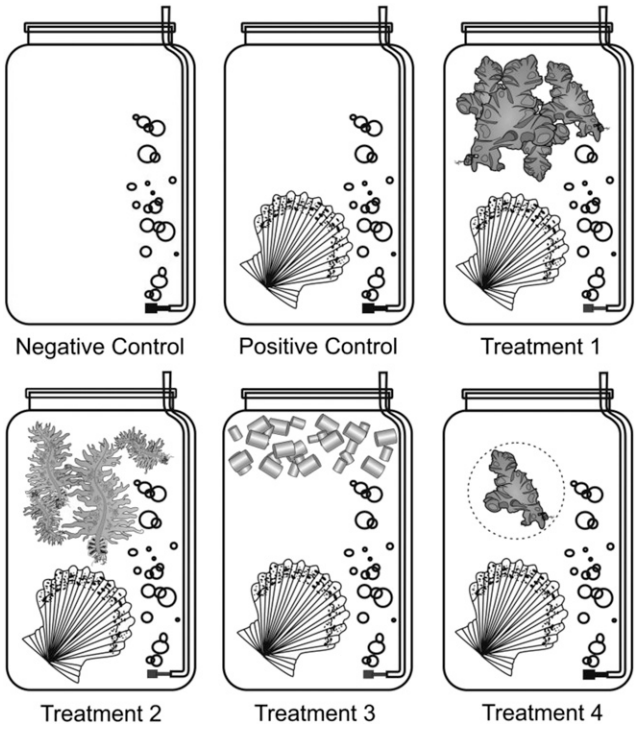

Figure 1. Schematic of the experimental setup (mean values of duplicate beakers are shown). Negative control: beaker without bivalve or filters; Positive control: scallop without any filtration; Treatment 1: scallop with *Ulva* spp. as biofilter, Treatment 2: scallop with *Undaria pinnatifida* as biofilter; Treatment 3: scallop with bacterial biofilter, and Treatment 4: scallop with mechanical filtration of the seawater during the first 8 days followed by addition of a biologic biofilter (*Ulva* spp.) for the remaining 6 days of the experiment.

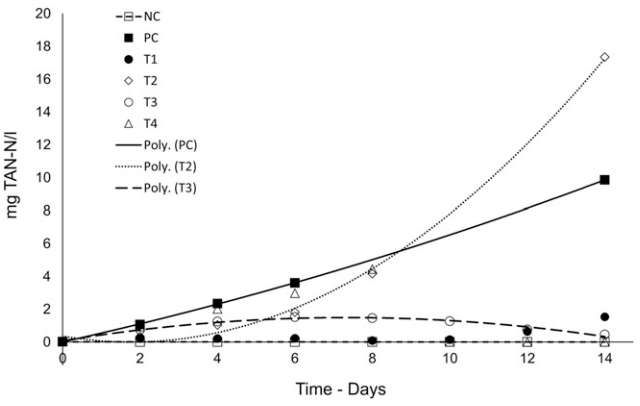

Figure 2. Buildup of TAN-N (mg L^{-1}) during the experiment. (Poly. indicates polynomial functions fitted to the data.)

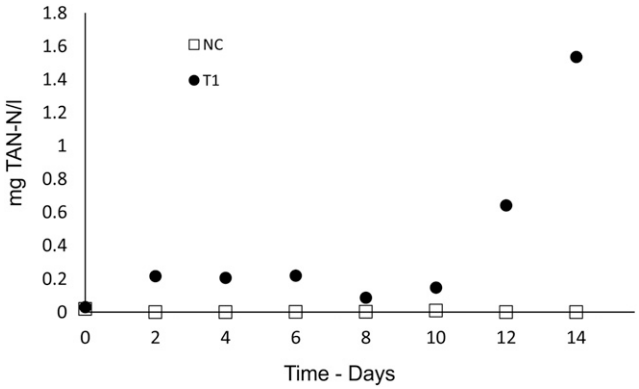

Figure 3. Buildup of TAN-N (mg L^{-1}) comparing NC: without bivalve or filters and T1: *Ulva* spp. used as biofilter.

this control were the lowest measured in all assays. In the PC, the TAN-N concentration increased during the experiment, reaching a final mean value of 9.85 mg L^{-1} at 14 days. The best fit to the data was obtained with a second-order polynomial regression model ($y = 0.004 + 0.525x + 0.013x^2$; $P < 0.001$, coefficient of determination, $R^2 = 0.99$; Wi: 0.668) (Fig. 2). A linear regression, however, also provided a good fit to the data ($0.713x - 0.331$; $P < 0.001$, $R^2 = 0.98$; Wi: 0.299; $\Delta i < 2$). Using the linear fit, the rate of TAN-N increase was estimated to be 0.03 mg L^{-1} h^{-1}.

In treatment T1 (with *Ulva* spp.), the TAN-N concentration remained between 0.02 and 0.22 mg L^{-1}, at levels below those measured in PC, T2, and T3 until the 10th day. After that, the TAN-N concentration increased, attaining a mean value of 1.54 mg L^{-1} on the 14th day (Fig. 3). Changes in the appearance and color of the algal biomass were observed at this time. Using PC concentrations as a reference, the removal percentages were Rd8 98% and Rd14 84% (Fig. 4).

In treatment T2 (with *Undaria pinnatifida*), the TAN-N concentration was 4.17 mg L^{-1} on the 8th day (Rd8 22.5%, Fig. 4); that measured on the 14th day was the highest recorded in all treatments and controls (mean value ± SE = 17.35 ± 0.40 mg L^{-1}). Changes in seaweed biomass appearance and in the color of the seawater were observed starting on the 4th day. A third-order polynomial regression provided the best fit to the data ($y = 0.042 + 0.097x + 0.0094x^2 + 0.005x^3$; $P < 0.001$, $R^2 = 0.99$; Wi: 0.603) (Fig. 2). A second-order polynomial regression, however, provided a comparable fit ($y = 0.036 - 0.41x + 0.115x^2$; $P < 0.001$, $R^2 = 0.99$; Wi: 0.394; $\Delta i < 2$) (Fig. 2).

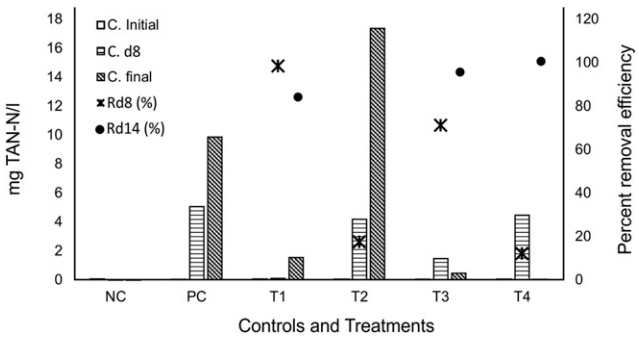

Figure 4. Mean TAN-N concentrations (TAN-N = NH_4^+-N + NH_3-N) in mg L^{-1}, on days 0 (Initial), days 8 and 14, presented as bars. The removal rates of TAN-N on the 8th day (% Rd8) and on the 14th day (% Rd14) are also indicated.

In treatment T3, bacterial biofilter treatment, the highest TAN-N concentration was measured on the 8th day with a mean value of 1.45 mg L^{-1} (Rd8 71%). It subsequently began to decline, reaching a mean value of 0.45 mg L^{-1} (Rd14 95%) on the 14th day (Fig. 4), which was lower than the levels measured in PC, T1, and T2. A third-order polynomial regression provided the best fit to the data ($y = -0.011 + 0.492x - 0.047x^2 + 0.001x^3$; $P < 0.001$, $R^2 = 0.97$; Wi = 0.9) (Fig. 2).

In treatment T4, during the mechanical filtration period (first 8 days), there was a linear increase in TAN concentration ($y = 0.545x - 0.1$; $P < 0.001$, $R^2 = 0.97$; Wi: 0.711), with a lower removal rate (Rd8 12%) than that recorded in T2. After *Ulva* spp. was added to the systems, the TAN-N concentration decreased to values as low as those measured in the NC control (Rd14 100%), a condition that persisted until the end of the experiment (Fig. 2).

Results obtained in the present study are compared with seawater quality standards and guidelines for aquaculture in Table 1. Table 2 summarizes the initial, day 8, and final TAN concentrations for controls and treatments and presents the corresponding estimates of NH_3 concentrations. The final TAN concentration measured in treatment T3 was close to the reported safety values.

DISCUSSION

In laboratory experiments, the concentration of metabolic TAN must be controlled because high concentrations of this compound could be toxic for marine organisms. Animal tolerance of TAN and excretion rates are influenced by several biological factors, such as the species, exposure time to a particular TAN concentration, starvation, the quality and quantity of food, and the physicochemical characteristics of seawater such as pH, temperature, and salinity (Bayne & Scullard 1977, Navarro & Gonzalez 1998, Navarro 2001, MacDonald et al. 2006, Soria et al. 2007, Jansen et al. 2012, Buzin et al. 2015).

In this study, the increase of TAN concentration in the PC was used to estimate an apparent excretion rate of 0.03 mg L^{-1} $N\text{-}NH_4^+$/h, which is of the same order of magnitude as that reported by Navarro and Gonzalez (1998) for the scallop *Argopecten purpuratus* (0.019–0.051 mg $N\text{-}NH_4^+$ L^{-1}/h). Epifanio and Srna (1975) found that oysters and clams were markedly resistant to high TAN concentrations and reported a sublethal level of 5.6 mg L^{-1} $N\text{-}NH_4^+$ for the oyster *Crassostrea virginica* (the test solution was prepared by dissolving reagent-grade ammonium chloride in seawater). In the present study, this concentration was only exceeded after the 8th day in PC and T2, but neither lethal nor sublethal effects were visually detected in the tested scallops. Furthermore, pH values recorded throughout all the assays (7.8–8.0) ensured the prevalence of the ionic form (NH_4^+-N) over the most toxic, nonionized compound (NH_3-N).

There is limited information about the safety level of nitrogenous compounds for seawater aquaculture of bivalves. As shown in Table 1, the recommended safety values are between 0.59 and 1.17 mg L^{-1} for TAN and between 0.1 and 0.2 mg L^{-1} for NH_3. In some cases, TAN and NH_3 concentrations were higher than these guidelines. Results of this study

TABLE 1.

Summary of selected seawater guidelines and seawater quality standards for aquaculture from the literature.

	TAN (mg L^{-1} TAN-N)	NH_3 (mg L^{-1} NH_3-N)	TAN (mg L^{-1} TAN)*	NH_3 (mg L^{-1} NH_3)†	TAN (μM L^{-1} TAN-N)
Trigger value PC95 ANZECC (2000)‡	**0.91**	0.01	1.17	0.01	65.00
Trigger value revised by Batley and Simpson (2009)	**0.46**	0.01	0.59	0.01	**32.86**
Chronic marine criterion USEPA (1989)	0.76	0.01	0.98	0.01	**54.30**
General aquaculture safety level—mainly for freshwater fish—revised by Lawson (1995)	0.78	0.01	**1.00**	**0.02**	55.43
Aquaculture safety level for marine fish by Huguenin and Colt (1989)	0.78	**0.01**	1.00	0.01	55.36
Aquaculture safety level for black tiger shrimp brood stock by Menasveta et al. (2001)	**0.50**	0.01	0.64	0.01	35.71

The values given by the various authors are boldfaced. NH_3 was estimated as 1.29% of TAN, for temperature (T) = 12°C, pH = 7.5–8, and salinity = 31–35 (see Zepka Baumgarten et al. 1996).

* Conversion factor from TAN-N: 1/0.776.

† Conversion factor from NH_3-N: 1/0.822.

‡ Value for 95% species protection (pH: 8; T: 20°C). Derived from chronic nondetectable effects after chronic (95 days) exposure.

suggest that the Tehuelche scallop is tolerant to TAN and NH_3 levels at least one order of magnitude higher than the reported safety levels. It is noteworthy, however, that high TAN concentrations could affect biological functions such as cell function (Cheng et al. 2004), lysosomal integrity (Fang et al. 2008), and Na-K-ATPase and Na-ATPase activities (Pagliarani et al. 2008). Potential chronic effects in Tehuelche scallops beyond the 14-day experimental period tested in this study remain unknown.

The biofiltration methods showed significantly different responses. The pattern observed with the bacterial biofilter (T3) is consistent with the development of nitrification. The removal efficiency of Rd14 95% of the bacterial biofilter was similar to that (98%) found by Seo et al. (2001). In the present study, it was also noted that the uptake rate by nitrifying bacteria in the biofilter was in decrease at the end of the experiment. This pattern could indicate slow stabilization of the experimental system (Bower & Turner 1981, Seo et al. 2001) or reduction of nitrifications due to the effects of pH. It is known that nitrification rate drops markedly with decreasing pH. Huesemann et al. (2002) found that, relative to what occurs at pH 8, nitrification rate in seawater is reduced by ca. 15% at pH 7.8, 50% at pH 7, and >90% at pH 6.5. Furthermore, these authors concluded that nitrification activity essentially ceases at pH 6. Nonetheless, the biological history and conditions under which bacteria grow in nature may affect their response to pH (Srna & Baggaley 1975). In this study, the bacterial composition was not evaluated, and it was assumed that the bacterial assemblage consisted of the same strains of bacteria found in the coastal marine environment of Puerto Madryn (Golfo Nuevo). It is possible that more resistant strains could have been selected during activation of the biofilter (Zobell & Anderson 1936). The presence of heterotrophic bacteria is also possible (Chen et al. 2006), although no reduction of DO was detected, suggesting the absence of competition by heterotrophic bacteria. Further studies on the nitrifier bacterial composition are required.

In closed marine systems, the pH is often reduced because the nitrification process by bacteria in filter beds results in the production of hydrogen ions and, on the other hand, because respiration by the organisms in the tank enhances CO_2 acidity (Spotte 1974). Throughout the present study, however, the pH

TABLE 2.

Comparison of concentrations of nitrogenous compounds (in mg L^{-1}) obtained in the present study with water quality standards and guidelines for aquaculture.

	TAN (mg/L TAN-N)			NH_3 (mg/L NH_3-N)			TAN (mg/L TAN)*			NH_3 (mg/L NH_3)†		
	Initial	d8	Final	Initial	d8	Final	Initial	d8	Final	Initial	d8	Final
NC	0.02	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00
PC	0.02	5.04	9.85	0.00	0.06	0.13	0.02	6.49	12.69	0.00	0.08	0.15
T1	0.02	0.09	1.54	0.00	0.00	0.02	0.04	0.11	1.98	0.00	0.00	0.02
T2	0.02	4.17	17.35	0.00	0.05	0.22	0.02	5.37	22.35	0.00	0.07	0.27
T3	0.02	1.45	0.45	0.00	0.02	0.01	0.02	1.87	0.58	0.00	0.02	0.01
T4	0.02	4.44	0.01	0.00	0.06	0.00	0.03	5.73	0.01	0.00	0.07	0.00

The NH_3 concentrations were estimated as 1.29% of TAN, for T = 12°C, pH = 7.5–8, and salinity = 31–35 (see Zepka Baumgarten et al. 1996). The underlined values indicate values that exceeded water quality standards.

* Conversion factor from TAN-N: 1/0.776.

† Conversion factor from NH_3-N: 1/0.822.

remained between 7.8 and 8, a range which is favorable to nitrification (Srna & Baggaley 1975). The experimental duration was probably short enough to maintain an optimum pH. Bivalves may also rely on the dissolution of calcareous shells to buffer pH changes (Poxton & Allouse 1982, Truchot 1990, Seibel & Walsh 2003). Further research should thus include control of not only pH but also alkalinity. If necessary, regular addition of soluble buffering agents, such as sodium bicarbonate or sodium carbonate should be considered (Bower & Turner 1981).

In treatment T2 with *Undaria pinnatifida* as biofilter, TAN concentrations exhibited a greater rate of increase and attained higher values than the ones in the PC control. This trend may be related to seaweed degradation that was observed during the experiment, probably because of the lack of vitamins and or other specific nutrients. In agreement with findings of Cahill et al. (2010), *Ulva* spp. (T1) was the more efficient biofilter than *U. pinnatifida*, the other seaweed tested. The higher TAN affinity of *Ulva* spp. with respect to *U. pinnatifida* has been previously reported using other nitrogenous sources (Torres et al. 2004, Gil et al. 2005). Gil et al. (2005) found up to 90% TAN removal from diluted sewage; Krom et al. (1995) reported 80% removal from a marine fish pound; and Schuenhoff et al. (2003) reported 30% removal, resulting in removal from only 50% of the volume. Increases in TAN concentration in T1 after the 10th day could be due to seaweed fragmentation. Similar results were found by Mao et al. (2009) for a system combining the scallop *Chlamys farreri* and seaweed *Gracilaria lemaneiformis* when they used seaweeds as biofilters in aquaculture.

Treatment T4 showed that filtration of settled organic matter on the bottom of the beakers (first 8 days), followed by *Ulva* spp. addition, could provide a useful complementary method of dissolved nitrogenous removal. Under these conditions, the seaweed was capable of reducing TAN concentrations from 4.44 mg NH_4^+-N L^{-1} (8th day) to undetectable levels by the 14th day. On the one hand, removal of sedimented organic matter would avoid ammonium buildup due to remineralization; on the other hand, shortening the algae's exposure to experimental conditions would prevent its degradation and further recycling of TAN.

Species-specific physiological aspects could be related to the differential performance of *Ulva* spp. and *Undaria pinnatifida* as biofilters. Results obtained in this study are in agreement with previous studies, which found that *Ulva* spp. from Golfo Nuevo has a higher affinity for ammonium than *U. pinnatifida* growing in the same area. The TAN uptake ability by seaweeds is conditioned by several biological factors, principally, individual age and natural history (Harrison et al. 1986, Campbell 1999, Chopin et al. 2001, Moustafa et al. 2014), as well as by physical factors such as the dissolved form of N. Generally, seaweeds can take up NH_4^+ more readily than oxidized forms of N (Lobban et al. 1985, Neori 1996).

CONCLUSIONS

Under the experimental conditions tested in this study, *Ulva* spp. from Golfo Nuevo provided a more efficient biofilter than *Undaria pinnatifida*. The former seaweed was able to improve water quality by decreasing the TAN concentrations within 2 days after its addition to a nutrient-rich seawater aquarium and by controlling TAN levels near recommended safety levels. The seaweed *Ulva* spp. would thus represent a good alternative for the traditionally used bacterial biofilter. It could be suitable in research studies of bivalves at a laboratory scale, as well as during acclimation periods.

This study contributes to the determination of the resistance of the scallop *Aequipecten tehuelchus* to high TAN concentrations. Results also provide information that can be useful to conduct experiments with a scallop species that represents an overexploited artisanal resource from the Patagonian coast.

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