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SHORT COMMUNICATION

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Abarenicola pusilla (Quatrefages, 1866): A novel species for fish waste bioremediation from marine recirculating aquaculture systems

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Due to the rapid growth of the aquaculture industry in recent decades, its environmental impact has become an important issue for its own sustainability. The release of suspended solids, particulate and dissolved organic matter (mainly from faecal production and feed wastage) induce oxygen depletion (Srithongouthai & Tada, 2017), eutrophication and biodiversity loss in aquatic environments (Cubillo et al., 2016). Organic enrichment from aquaculture activities is mainly caused by increases in organic nitrogen (N), carbon (C) and phosphorus (P) compounds in adjacent water bodies and sediment (Granada, Sousa, Lopes, & Lemos, 2015; Marinho-Soriano et al., 2011).

In addition, aquaculture effluent discharges may be associated with other compounds such as pathogens, heavy metals, hormones or antibiotics, thus generating a risk for human health (Madariaga & Marín, 2016; Muziasari et al., 2016).

Recirculation aquaculture systems (RAS) allow cultivation of aquatic organisms on land with a percentage of water exchange less than 1% per day (Orellana, Waller, & Wecker, 2014). RAS offer greater effluent discharge control, while also allowing easier recycling of compounds generated in the process (Martins et al., 2010). Treatment and disposal of organic sludge from RAS (which is of lower volume and is more concentrated than sludge generated by open aquaculture systems) has subsequently become a logistical challenge for the aquaculture industry. Its high water content, with preliminary percentages around 90%, requires additional treatment (Aruety et al., 2016). The salt content of organic sludge from marine RAS limits its use as soil fertilizer (Zhang, Spanjers, & van Lier, 2013) and decreases the efficiency of anaerobic digestion systems for methane production (Luo, Ma, Li, Tan, & Liu, 2015), where these are most common uses for sludge treatment from aquaculture on land.

Implementation of bioremediation organisms in marine RAS effluents is a strong response to this challenge. Species of marine polychaetes such as *Sabella spallanzanii* (Pierri, Fanelli, & Giangrande, 2006), *Capitella* sp. (Kinoshita et al., 2008), *Nereis diversicolor* (Pajand, Soltani, Bahmani, & Kamali, 2017), *Nereis virens* (Brown, Eddy, & Plaud, 2011), *Perinereis nuntia* (Palmer, 2010) and *Arenicola loveni loveni* (Yearsley, Jones, Britz, & Vine, 2011) have shown their efficacy in removing and assimilating organic nutrients released from aquaculture production.

This study was performed to evaluate organic matter removal in a marine RAS effluent using *Abarenicola pusilla* (Quatrefages, 1866), a polychaete present in Chilean coasts and estuaries. The experiments were conducted using yellowtail kingfish (*Seriola lalandi*, Cuvier & Valenciennes, 1833) marine RAS sludge. RAS was operated with 310 individuals of yellowtail kingfish with an average weight ($\pm SD$) of 456.20 $\pm$ 54.30 g and a stocking density of 30.1 kg m^{-3}. Sludge was removed from the drum filter of the RAS (HEX F1-1) equipped with a 60 μ screen panel and was collected 24 hr before trials. The results of the sludge characterization are shown in Table 1.

Sixty individuals of *Abarenicola pusilla* (2.73 $\pm$ 0.44 g) were transported alive from the Laboratory of Marine and Limnological Sciences (UaCh, Valdivia, Chile) in a wet plant substrate. They were maintained for 4 hr in filtered seawater at open flow to eliminate possible stomach contents (purging process). Fifteen individuals were dehydrated in a muffle furnace at 333.15 $\pm$ 5 K until constant weight to determine their water content (88.58% $\pm$ 3.14%). The remaining individuals were implemented in the experimental system and a period of 48 hr was established for acclimatization purposes (Weston, Penry, & Gulmann, 2000).

TABLE 1 Sludge characterization of yellowtail kingfish marine recirculating aquaculture systems (RAS) used for the experiments (mean values ± *SD*, *n* = 5)

pH	7.28 ± 0.01
Temperature (K)	294.95 ± 0.10
O_2 (% saturation)	69.85 ± 1.44
O_2 (mg/L)	4.93 ± 0.10
Total ammonia nitrogen (TAN) (mg/L)	1.95 ± 0.13
Nitrite-nitrogen (NO_2^- - N) (mg/L)	0.48 ± 0.02
Nitrate-nitrogen (NO_2^- - N) (mg/L)	12.00 ± 0.22
Salinity (psu)	35.00 ± 0.50
Water content (%)	89.91 ± 0.11
Total nitrogen (%)	2.11 ± 0.001
Total carbon (%)	17.25 ± 0.01
C/N ratio	8.17

Total Carbon and Total Nitrogen were determined using an Isotope Ratio Mass Spectrometer, IRMS, Thermo Delta Advantage associated to Elemental Analyzer Flash EA2000.

Sixteen sand filtration beds were implemented and ordered into four series. A substrate layer with a depth of 5 cm (grain size <0.5 mm) was implemented in each bed (Crane & Merz, 2012) and was transformed into inert material after incineration (723.15 ± 50 K for 24 hr) (Bischoff, 2007). Above the substrate, a layer of 4 cm of filtered seawater (pH = 7.74 ± 0.19; T^a = 291.7 ± 1.24 K; 36 psu) permanently covered the experimental units (Görlitz, 2012). Marine RAS sludge was added as 0.5%, 2%, 4% and 10% for each of the series of experimental beds, relative to the total inert substrate content. *Abarenicola pusilla* individuals were added at a rate of 60 individuals per m^2 in all units. Controls were carried out as replicates without polychaetes.

During the 28 days of trials, the total ammonia nitrogen (TAN, Nessler method), nitrite-nitrogen (NO_2^-, diazotization method), nitrate-nitrogen (NO_3^-, cadmium reduction method) were measured daily by analytical methods for dissolved inorganic nutrients using a spectrophotometer (Hach DR-3900) to determinate concentration and excretion rate by the polychaetes. Abiotic parameters, such as oxygen concentration, pH and temperature were also recorded on a daily basis using a portable multimetre probe (Hach HQ40D). These results are shown in Table 2. To determinate total organic matter (TOM) removal by the polychaetes, TOM content was determined by calculating the difference in the dried sediment before and after application in a muffle furnace at 723.15 ± 50 K for 24 hr. Differences in total Carbon and total Nitrogen were determined using an Isotope Ratio Mass Spectrometer (IRMS, Thermo Delta Advantage) associated to Elemental Analyzer (Flash EA2000). Specific growth rate, measured using differences in dry weight before and after the trials, and survival performance of the *Abarenicola pusilla* were also studied. Observed production - biomass ratio (P/B ratio) was also calculated as a good indicator of biomass production in marine macrobenthos species. Total mortality was determined as the difference between the number of initial and recovered organisms. SPSS v.19 software was used to perform all statistical analyses. The normality and homogeneity of the obtained data were tested by the Shapiro–Wilk test prior to analysis. One-way ANOVA was used to determine the differences between the growth rate of the polychaetes, TAN excretion rate and TOM removal at each added sludge percentage, and to compare these with the control samples. Significant differences were identified using Tukey's tests. All data were given as mean ± standard deviation (*SD*) and the differences were considered as significant at $p < .05$.

Two types of assays were performed in terms of water recirculation. In trials without a water change, no growth or TOM removal was observed and the highest survival performance was 16.19% ± 14.66% of individuals after 6 days of experiments. Anoxic sediment and water turbidity were observed in all systems 48 hr after starting the trials.

Results of growth performance and survival of *Abarenicola pusilla* are presented in Table 3. Assays with 100% water change every 24 hr achieved a maximum growth rate of 123.56 ± 20.12 g m^{-2} day^{-1} at 0.5% added sludge. This means a specific growth rate (μ) of 8.90 day^{-1}. Maximum P/B ratio of *Abarenicola pusilla* in this study was 47.85 ± 11.86 $year^{-1}$, calculated according to Tumbiolo and Downing (1994). The survival performance was highest in beds with 2% added sludge, which resulted in 83.33% ± 28.86% of alive individuals at the end of the experiments. These results can be related to an adequate availability of organic matter before poor water quality and sediment conditions appear in the bed filters.

Previous studies with other species of polychaetes obtained biomass increases of 2.2% per day for *Abarenicola pacifica* in laboratory trials (Taghon & Greene, 1990), 3.40% for *Nereis diversicolor* fed with *Huso huso* waste (Pajand et al., 2017), 1.66% for *Perinereis nuntia*

TABLE 2 Abiotic parameters measured after 24 hr in samples with *Abarenicola pusilla* for all sludge concentrations (mean values ± *SD*, 28 days of trials)

	0.5% sludge	2% sludge	4% sludge	10% sludge
pH	7.64 ± 0.23	7.73 ± 0.21	7.70 ± 0.24	7.71 ± 0.26
O_2 (% saturation)	93.88 ± 4.10	93.71 ± 2.37	92.44 ± 3.84	80.72 ± 8.84
Temperature (K)	292.26 ± 0.56	292.29 ± 0.55	292.31 ± 0.50	292.31 ± 0.49
NO_2- N (mg/L)	1.19 ± 1.13	0.97 ± 0.59	2.65 ± 1.71	2.23 ± 1.71
NO_3- N (mg/L)	2.52 ± 3.21	2.68 ± 2.96	3.25 ± 3.13	4.84 ± 4.75

Initial water, NO_2 - N = 0.02 ± 0.01 mg/L; NO_3 - N = 0.54 ± 0.16 mg/L; pH = 7.64 ± 0.17; O_2 = 99.41% ± 3.66%; Temperature = 291.15 ± 1.13 K.

TABLE 3 Growth parameters and survival rate of *Abarenicola pusilla* for all sludge concentrations (mean values ± *SD*, *n* = 60, 28 days of trials)

	0.5% sludge	2% sludge	4% sludge	10% sludge
Absolute growth rate (g m^{-2})	123.556 ± 20.121	96.074 ± 46.532	86.165 ± 30.337	53.256 ± 34.727
Specific growth rate (μ) (day^{-1})	8.902 ± 1.536	6.757 ± 2.632	6.236 ± 2.002	3.936 ± 2.435
Survival rate (%)	66.666 ± 28.868	83.333 ± 28.868	55.555 ± 9.623	35.555 ± 3.849
P/B ratio ($year^{-1}$)	47.855 ± 11.864	33.894 ± 17.010	30.178 ± 12.613	17.652 ± 11.658

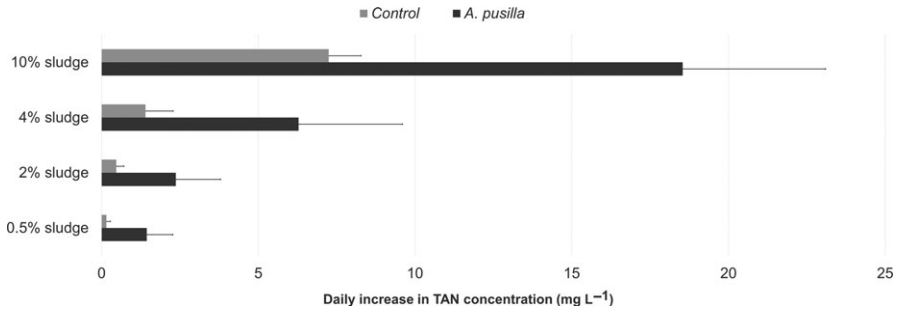

FIGURE 1 Daily increase in TAN concentration (mg/L) measured for *Abarenicola pusilla* and control samples (mean values ± *SD*, *n* = 28). Significant differences ($p < .05$) were found in sand filtration beds at 4% and 10% of added sludge comparing to other concentrations and also with their corresponding controls. No differences were found in TAN increase between *A. pusilla* filters at 0.5% and 2% sludge concentrations

vallata with flounder faeces (Honda & Kikuchi, 2002) and 9.34% for *Sabella spallanzanii* implemented in aquaculture effluents (Granada et al., 2015). In this study, *Abarenicola pusilla* achieved a maximum increase in 12.92% of polychaete biomass per day.

TAN concentration was analysed as an important factor causing high levels of mortality in laboratory tests using other groups of polychaetes (Bischoff, 2007). The maximum increase in TAN concentration per day was 18.54 ± 4.56 mg/L in the assays with 10% added sludge (Figure 1). This means an excretion rate of 0.139 ±0.043 mg g^{-1} day^{-1} (Figure 2), less than 0.25 mg g^{-1} day^{-1} found by Honda and Kikuchi (2002) for *Perinereis nuntia vallata* fed on sludge of *Paralichthys olivaceus*. Due to organic enrichment, TAN increase was also observed in the control samples (Mook et al., 2012). The decrease in oxygen concentration was in agreement with the percentage of sludge added, dropping 1.62 ± 0.95 mg/L (23.21% ± 10.55% saturation) per day in the 10% sludge samples.

TOM removal was achieved at all sludge concentrations (Figure 3). In the control samples, an increase in TOM concentration was observed. Due to the high concentration of organic matter, degradation of sediment quality was observed as it presented a blackish mud layer on the surface after 48–72 hr of testing in the 10% sludge samples, similar conditions found under aquaculture farming cages (Srithongouthai & Tada, 2017). The highest removal efficiency was 83.96 ± 7.56 g of TOM removed per m^2 and day at 4% added sludge, meaning 0.71% of total TOM removed daily. The percentage of total N and C removed in these trials was, respectively, 0.72% and 8.02%, which means 5.81 and 71.10 μg per day.

These results demonstrate that *Abarenicola pusilla* is a good potential candidate for organic matter reduction in fish waste from a marine recirculation system. Due to better survival rate and MOT removal, 2% of added sludge should be recommended for trials of this type. The implementation of a biofiltration system could enhance these results, decreasing TAN concentration and improving sediment quality and water oxygenation. More research is needed to determinate the performance of this species on a remediation system with these characteristics using marine aquaculture sludge.

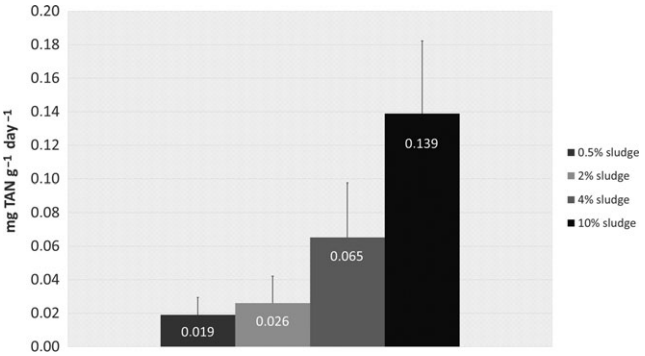

FIGURE 2 TAN excretion rate of *Abarenicola pusilla* during the experiments. Values are shown as mg of TAN excreted per gram of individual and day (mean ± *SD*, *n* = 28). Significant differences ($p < .05$) were found for 10% sludge units comparing to the other systems

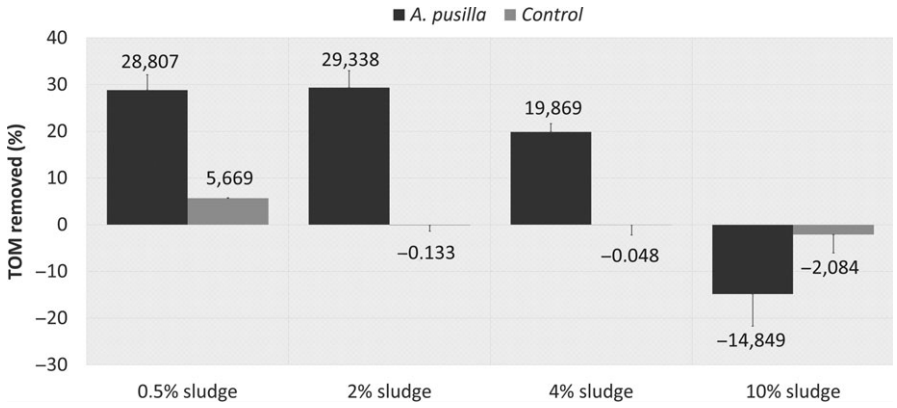

FIGURE 3 Total percentage (%) of TOM removed during the trials. *Abarenicola pusilla* performance is shown in black columns. Grey columns represent control units (mean values ± *SD*, 28 days). Significant differences ($p < .05$) were found between sand filtration beds at 0.5%, 2% and 4% of added sludge and their corresponding controls. No differences were found in TOM removal of *A. pusilla* filters at these sludge concentrations

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