



# Corrigendum: Spatial Patterns of *Thalassia testudinum* Immune Status and *Labyrinthula* spp. Load Implicate Environmental Quality and History as Modulators of Defense Strategies and Wasting Disease in Florida Bay, United States

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**Keywords:** immunocompetence, host-pathogen interactions, hyposalinity stress, opportunistic pathogens, environmental fluctuation, anthropogenic influences, resistance, tolerance

## OPEN ACCESS

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### Specialty section:

This article was submitted to  
Marine and Freshwater Plants,  
a section of the journal  
Frontiers in Plant Science

**Received:** 17 February 2022

**Accepted:** 25 February 2022

**Published:** 21 March 2022

### Citation:

Duffin P, Martin DL, Furman BT and  
Ross C (2022) Corrigendum: Spatial  
Patterns of *Thalassia testudinum*  
Immune Status and *Labyrinthula* spp.  
Load Implicate Environmental Quality  
and History as Modulators of Defense  
Strategies and Wasting Disease in  
Florida Bay, United States.  
*Front. Plant Sci.* 13:877673.  
doi: 10.3389/fpls.2022.877673

## A Corrigendum on

**Spatial Patterns of *Thalassia testudinum* Immune Status and *Labyrinthula* spp. Load Implicate Environmental Quality and History as Modulators of Defense Strategies and Wasting Disease in Florida Bay, United States**

by Duffin, P., Martin, D. L., Furman, B. T., and Ross, C. (2021). *Front. Plant Sci.* 12:612947.  
doi: 10.3389/fpls.2021.612947

In the original article, there was an error. The primer set sequences we originally provided did not accurately reflect the primer set sequences we used to conduct the study. The original study referenced, however, does contain the correct sequence (Duffin et al., 2020).

A correction has been made to *Materials and Methods* > *Labyrinthula* spp. Load > *Quantitative Real-Time PCR Procedure* > *qPCR Protocol*, paragraph one:

Primers (LabPathITS1-3F: 5'-CAA CTC AAT GAA TAT CTT GGT TTC C-3', and LabPathITS1-3R: 5'-CCG CTT ATT GAT ATG CTT AAA TTC-3') targeted the ITS region of the ribosomal RNA gene complex (Duffin et al., 2020). Quantitative PCR (qPCR) reactions were prepared with the following final concentrations: 1 ng  $\mu\text{l}^{-1}$  of DNA template, 0.025  $\mu\text{M}$  of each primer, 2.7 ng  $\mu\text{l}^{-1}$  of BSA, 1X of iTaq SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA), and nuclease free water up to 20  $\mu\text{l}$ . Reactions were run in triplicate on a CFX Connect thermal cycler (Bio-Rad) with the following cycle parameters: 5 min at 95°C, followed by 45 rounds of 30 s at 95°C and 60 s at 63°C. Reactions were terminated with a melting curve analysis (65–95°C, at 0.5°C increments). Results are reported as the number of *Labyrinthula* spp. cells per mg starting seagrass tissue (dry weight, ~5 mg). See Duffin et al. (2020) for additional details.

In the original article, there were a few errors in reporting concentrations of *Labyrinthula* strains used in the qPCR specificity assays. Specifically, there were three locations within a single sentence where “ml” was inadvertently used instead of “ $\mu\text{l}$ .”

A correction has been made to *Materials and Methods* > *Labyrinthula spp. Load* > *qPCR Strain Specificity*, paragraph two:

First, summary information was compiled for the average cycle quantification value (C<sub>q</sub>, also referred to as C<sub>t</sub>) of amplified strains across previous qPCR runs (conducted before and after publication of the Duffin et al., 2020 pilot study) with varying DNA template concentrations, to assess whether the qPCR assay amplified putatively pathogenic isolates more readily (i.e., at a lower C<sub>q</sub> value, on average) than non-pathogenic isolates. In the pilot study, we tested strains using very high concentrations of DNA isolated from pure *Labyrinthula* sp. cultures. Thus, secondly, we adjusted the concentration of starting *Labyrinthula* sp. template DNA to 25 cells per reaction (converted from *Labyrinthula* sp. cells per mg dry seagrass tissue), to better match realistic concentrations in the field. This reflects a cell count greater than the load present in >95% (and within 1 SD of the highest load detected) of our FB-collected *T. testudinum* samples with detectable levels of the pathogen (this study). Third, we accounted for the possibility that non-specific binding may occur when using pure non-pathogenic *Labyrinthula* sp. cultures

as the only template, so we introduced UltraPure™ Salmon Sperm DNA Solution (Invitrogen™) as background DNA in our diagnostic qPCR assays (i.e., to mimic host “background” DNA). Fourth, we evaluated bias for the case of pathogenic types being outnumbered by non-pathogenic. Specifically, we tested pathogenic isolate “8b” and non-pathogenic isolate “98b,” both of which reliably amplified in Duffin et al. (2020), but with notably different melt curve peaks at 76°C and 78.5°C, respectively, under several template DNA conditions: (1) pure *Labyrinthula* sp. culture at 1x concentration (2 μl 8b at 1x ≈ 646.8 cells per reaction; 2 μl 98b at 1x ≈ 1522.5 cells per reaction); (2) *Labyrinthula* sp. DNA equivalent of 25 cells per reaction with salmon sperm DNA to bring template volume to 20 ng total (i.e., 1 ng/μl reaction volume); and (3) both *Labyrinthula* sp. DNA loaded in a single reaction at a 1:70 ratio (~5.36 cells of 8b; ~375.2 cells of 98b per reaction), brought to 20 ng total with salmon sperm DNA. Each reaction was run in duplicate.

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

## REFERENCES

Duffin, P., Martin, D. L., Pagenkopp Lohan, K. M., and Ross, C. (2020). Integrating host immune status, *Labyrinthula* spp. load and environmental stress in a seagrass pathosystem: assessing immune markers and scope of a new qPCR primer set. *PLoS One* 15:e0230108. doi: 10.1371/journal.pone.0230108

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