EFFECTS OF TEMPERATURE, SALINITY AND DIET ON GROWTH, SURVIVAL AND FATTY ACID COMPOSITION OF THE RAGWORM *HEDISTE DIVERSICOLOR* (OF MÜLLER, 1776) (ANNELIDA: NEREIDAE)

A. Hagemann[1]*, A. Villena-Rodríguez[2], O. Monroig[2], J.C. Navarro[2], L. Filipe C. Castro[3,4] and A. M. Malzahn[1]

[1]SINTEF Ocean, 7465 Trondheim, Norway. Email: andreas.hagemann@sintef.no. [2]Instituto de Acuicultura Torre de la Sal (IATS), CSIC, 12595 Ribera de Cabanes, Castellón, Spain. [3]CIIMAR /CIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros de Leixões. Av. General Norton De Matos s/n 4450-208 Matosinhos, Portugal; [4]Department of Biology, Faculty of Sciences, University of Porto, Rua do Campo Alegre 1021/1055, Porto, Portugal

Introduction

The common ragworm *Hediste diversicolor* is an omnivorous, burrowing polychaete with a broad tolerance to environmental conditions such as temperature, salinity and oxygen levels. This nutritious annelid has received a great deal of attention lately for its potential as an extractive species in IMTA systems to recapture high-value compounds from fed aquaculture particulate side streams (sludge) and its suitability for subsequent utilisation as a novel, omega-3 rich aquafeed ingredient (Wang et al., 2019). It has recently been shown that this species has the capacity for endogenous production of omega-3 long-chain polyunsaturated fatty acids (Kabeya, et al., 2020), however it is yet not understood how environmental cues affects this ability. We conducted two sets of experiments to assess the combined effects of diet, temperature and salinity on growth, survival and total body fatty acid composition in *H. diversicolor* juveniles.

Materials and Methods

Polychaetes (*H. diversicolor*) were collected from Leangen Bay, Trondheim, Norway (63°26′24.5″N, 10°28′27.7″E). To investigate the effects of diet and temperature on growth, survival and fatty acid (FA) composition of *H. diversicolor*, worms were fed mixes of solid biogas digestate (SBD) and salmon aquaculture sludge (SS) along a 4-step feed gradient ranging from pure SBD to pure SS, and a 5-step temperature gradient ranging from 5.8 to 17.1 °C, for 15 days, using fish feed as a control (Experiment 1). A second experiment was conducted to investigate the effects of salinity and temperature on the same variables. Here, the worms were fed the diet which yielded the highest growth rates in the first experiment (33:66 % SS:SBD) along 5-step salinity and temperature gradients ranging from 5 to 40 ppt and 7.7 to 17.9 °C, respectively, for a duration of 28 days. In both experiments, worms were fed isonitrogenous diets equalling 30 % of the worms' total body nitrogen per day (Wang et al., 2019). Both experiments were conducted in a temperature gradient table (modified after Thomas et al. (1963)) using a 18h:6h light:dark cycle. Worms (n=7-8) were stocked in glass beakers (800 mL) containing an eight cm thick layer of sand and sand- and bagfiltered (1 µm) seawater from the Trondheim fjord collected at 60 m depth. The worms were allowed to evacuate their guts in clean seawater for minimum 4 hours before each sampling and weighing. Water exchange and feeding was conducted every second day. Data analyses were performed using the inbuild statistical package of SigmaPlot v. 14.5.

Results and Discussion

In the first experiment, the highest specific growth rate (SGR) was found for worms fed a 33:66 % mix of SS:SBD (SGR= 0.025) at 14.7 °C. Growth was found to be significantly influenced by temperature (two-way ANOVA; $p<0.001$) and not by diet (two-way

ANOVA; $p>0.05$) (Figure 1A). Growth was only positively related to temperature up till 14.7 °C, whereafter SGR declined. The proportions of SS and SBD in the diet showed no significant effects on SGR within temperatures. Survival was high for all treatments combined (88±13 %), and both temperature and diet had significant effects on survival (two-way ANOVA; $p<0.001$ and $p>0.05$, respectively); mortality increased with increasing temperature, whereas feed type did not have such a pronounced, yet significant, effect on survival. In the second experiment, the highest specific growth rates (≤0.015) were found for worms maintained at high temperatures (≥14.3 °C) and high salinities (≥35 psu) (Figure 1B). Survival averaged 80.5±16.2 % for all treatments and was, opposed to growth rate, not significantly affected by neither salinity (One-Way ANOVA, Holm-Sidak *post hoc*; $p>0.05$) nor temperature (Kruskal-Wallis One Way Analysis of Variance on Ranks; $p= 0.380$).

No temperature- nor salinity-driven segregation patterns could be identified in the FA profiles of the worms, however a clear diet-driven segregation was found between worms fed lipid-rich fish feed (control) and lipid-poor SS and SBD diets (data not shown). Hence, our results indicate that the major influence on fatty acid composition in *H. diversicolor* is diet. We here demonstrated that short-term (≤4 weeks) alterations of environmental parameters have neglectable effects on the lipid profile in wild caught *H. diversicolor* juveniles (Villena-Rodríguez, A. et al., *in prep*, data not shown)

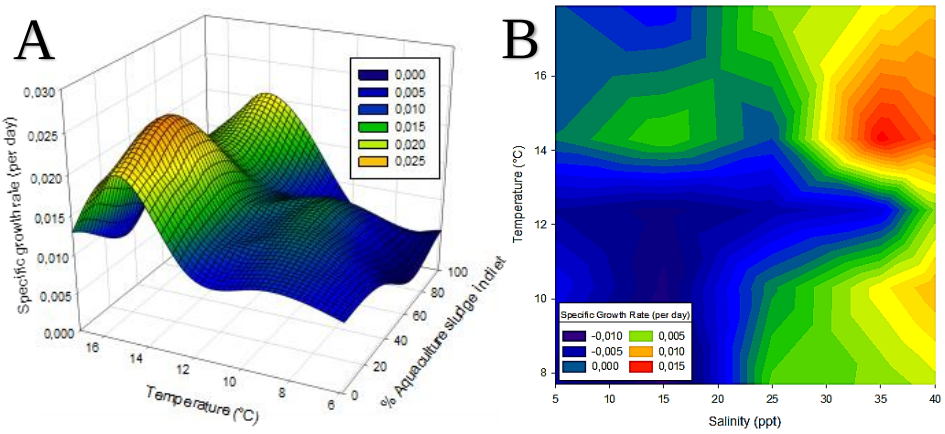

Figure 1. Specific growth rate (SGR) for *H. diversicolor* fed different ratios of solid biogas digestate (SBD) and salmon sludge (SS) at different temperatures over a 15-day period (A), and *H. diversicolor* fed the same diet at different temperatures and salinities over the course of 28 days (B).

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