Controls on Development and Nucleic Acid Indices in Tropical Planktonic Copepods

Thesis submitted by
Luiz Felipe Mendes de GUSMÃO M.Sc. (USP—Brazil)
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"*I often towed astern a net made of bunting, and thus caught many curious animals*"

Charles Darwin (The Voyage of the Beagle, 1839)

This thesis is dedicated to Débora

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Abstract

Copepods are some of the most abundant animals on the planet, accounting for a large proportion of mesozooplankton biomass in the oceans. They are an important link between microbial processes and higher trophic levels, and for this reason the measurement of copepod productivity is a central theme in Biological Oceanography. Because biological production is a function of growth rate, considerable effort has been invested in developing techniques for studying various aspects of copepod development and growth. Historically, most studies investigating the biology, growth and production of copepods have been conducted in temperate waters, and information on tropical copepod species is extremely scarce. Tropical species are often exposed to sub-optimal trophic resources and have higher metabolism expenditure than temperate species. These differences limit the direct application of current empirical models, based mostly on temperate data to the tropics. However, tropical seas comprise a large part of the world's oceans, and more studies on the biology of tropical copepods are necessary for a better understanding of biogeochemical cycles in the tropics, and for improved estimates of energy flow in the oceans. This thesis tries to fill this gap by advancing our understanding of some important characteristics of the development and growth of tropical copepods. The objective of this thesis is to determine the effectiveness of the application of traditional and biochemical indices for the study of growth and development of tropical copepods. More specifically, this study investigates the applicability of the artificial cohort and egg production techniques, and nucleic acid indices for the study of tropical copepod species.

In the first part of the thesis I used the artificial cohort technique to investigate trophic influences on the development, growth, production, and the occurrence of intersexes and skewed sex ratios in the tropical paracalanid copepod *Acrocalanus gracilis* from the Timor Sea. An artificial cohort of *A. gracilis* was followed from egg to adulthood, and its development characterised by the calculation of stage specific duration, median development time, moulting period duration, and moult and growth rates. In addition, the morphometry, sex ratios and the occurrence of intersexes in copepodite and adult stages was quantified during the development of the experimental population of *A. gracilis* and compared to the wild population. The food environment in the experimental incubation was poorer than in situ. The somatic growth of *A. gracilis* was log-linear throughout most of its development, even under variable and limited food conditions. Linearity of growth appears to be maintained at the cost of varying the moulting rate and stage duration. The development of this species conforms to the equiproportional

model, and in this respect is similar to other paracalanid species. The quality and quantity of food significantly affected the morphometry and body size of all copepodite stages of *A. gracilis,* indicating that trophic conditions play an important role in controlling the development features of paracalanids more generally. Moreover, intersexes were more frequent in the food limited experimental incubation than in the field. Adult males were present in the field, but were completely absent from the experiment. The sex ratios calculated considering intersexes as males in C5 and adults showed no significant difference between populations in the field and in the experiment. On the other hand, sex ratios in the experiment were significantly different from the field when intersexes were added to females. These results suggest that the occurrence of intersexes is a result of the process of sex change in male copepodites, and that these Intersexes can have a major effect on the sex ratio of copepod populations. The quality and quantity of food that the animals experience during development appears to be the key factor controlling sex change in copepods. The point of sex switching occurs late in the development of *A. gracilis*, and appears to be triggered by the nutritional condition of copepodite 4 males. Sex determination in copepods appears to be under strong environmental control, and sex change is an important mechanism determining the adult sex ratio of copepod populations, explaining the often female skewed sex ratios observed in copepod populations. In addition, the results and hypothesis presented here enhance our understanding and raise new questions about the sex determination and sex change processes in copepods, implications for sex ratios, and ultimately for the maintenance and success of copepod populations in nature.

In the second part of the thesis I evaluated the individual and interactive effects of food quantity, food quality and temperature on the egg production, nucleic acid content and nucleic acid—egg production relationship of the copepod *Acartia sinjiensis*. The egg production rate and nucleic acid content of *A. sinjiensis* females were quantified in a series of laboratory experiments where different food types (*Tetraselmis chuii*, *Pavlova salina*, *Isochrysis aff. galbana* and *Chaetoceros muelleri*) were offered at various concentrations and temperature levels. Food quality, quantity, temperature and their interaction significantly affected the nucleic acid content, egg production rate and the nucleic acid—egg production relationship of *Acartia sinjiensis*. The egg production, RNA:DNA ratio and individual RNA content of food-limited females were lower than animals fed non-limiting algae concentrations. Under non-limiting food conditions, *A. sinjiensis* females produced more eggs and had higher nucleic acid concentrations when fed *Tetraselmis* and *Pavlova*, than when fed *Isochrysis* and *Chaetoceros*. Under

food-limiting conditions, differences in food quality became evident as only the higher quality algae *Pavlova* and *Tetraselmis* could support egg production. Moreover, the relationship of RNA content, RNA:DNA ratio and egg production with food concentration followed the same sigmoid function up to an asymptotic (saturation) level. The minimum food concentration necessary to saturate the RNA content, RNA:DNA ratio and egg production was between 500—1000μg Cl^{-1}, as is the case in other *Acartia* species. Furthermore, the slopes of linear regressions using nucleic acid indices as predictors of egg production were similar in females fed different algal species (i.e. different food quality), but the intercepts of these regressions differed significantly. The DNA content of *A. sinjiensis* females was significantly affected by food and temperature, suggesting that it is not a good index of cell number in copepods. Despite this, the RNA:DNA ratio was as good a predictor of egg production as total RNA content in this species. While temperature appeared to have a minor effect on the egg production of *A. sinjiensis*, total, C- and N-specific nucleic acid indices had a strong negative correlation with temperature. Furthermore, the slopes of the regression lines of RNA content and RNA:DNA ratio as predictors of egg production increased non linearly with temperature. Finally, the interaction terms of RNA based nucleic acid indices with temperature were better predictors of egg production in linear models than these indices alone in *A. sinjiensis*. These findings indicate that temperature, food quantity and quality are key factors controlling the egg production rate, the nucleic acid content, and the nucleic acid—egg production relationship in *Acartia sinjiensis*. Finally, these results provide key information for the development of predictive models of egg production based on nucleic acid indices for *A. sinjiensis* and other tropical copepods.