17. ARE MALES SPECIMENS MORE ADEQUATE TO DETECT ANTROPOGENIC IMPACTS?

Chapter Objectives

To demonstrate sex effects on different aquatic species condition and its susceptibility to anthropogenic impacts.

Field sampling (photo Ecoreach)

EH principle: **1** – use of biota as impact indicators

INTRODUCTION

Estimates of the condition of aquatic organisms can be used to monitor the health or recovery of aquatic areas, under the ecohydrologic approach (Zalewski 2000, Chicharo *et al.* 2001). The ability of aquatic organisms to cope with environmental stress may be expensive in terms of energy and this cost of tolerance have negative counterparts in growth, reproduction, recruitment, susceptibility to disease, predation and physical disturbance (Jackson *et al.* 2002, Lloret *et al.* 2003, Oliva-Paterna *et al.* 2003). Density-dependent factors such competition and aggression can influence fitness, growth, reproduction, and survival (Hensor *et al*. 2005, Leitão 2006). **Indices of the condition of organisms are valuable for managers of aquatic ecosystems for assessment of the health status of populations (Brown, Austin 1996).**

ELABORATING OF THE EXPERIMENT

1. General description

The relationship between the condition of adults during the months prior to spawning and the number of recruits in the following year was been significant and positive for some aquatic species (Carbonell *et al.* in press). This relationship was stronger when only male condition was considered, suggesting that males must be considered differently (Carbonell *et al*. in press). Also Chicharo *et al*. (2007) showed that the males of three different marine species are more susceptive to environmental changes.

Nevertheless, there is a paucity of data on the effects of sex on growth, energetics, and condition of aquatic organisms. Several studies of the growth rates and conditions of aquatic organisms assumed no differences between males and females in the condition based on morphometric (Gerritsen, McGrath 2007) and on biochemical content of muscle tissue or of the whole organism eg.:Regnault and Luquet (1974), Paon and Kenchington (1995), Chícharo *et al.* (2003), and Norkko *et al.* (2005).

There are several methods to determine aquatic organisms condition, some of the most generalized are: the morphometric condition index (Nash *et al.* 2007), an indicator of the general well-being, this index assumes that heavier organism for a given length are in better condition, and the RNA/DNA ratio, this index is based on the assumption that the amount of deoxyribonucleic acid DNA, the primary carrier of genetic information, is stable under changing environmental situations, whereas the amount of ribosomal ribonucleic acid RNA is directly involved in protein synthesis, is affected by environmental changes (Bulow 1970).

The aim of this work is to quantify differences between male and female condition of fish and aquatic invertebrate's species, using biometric biochemical analysis.

2. Experimental design

It should be selected species with different habitats and feeding habits to negate the confounding effect of physiological, morphometric, and behavioural changes on differences in condition indices, between males and females. It can be suggested species with wide distribution such as gobiid fishes (eg *Pomatoschistus* spp), crustaceans (eg *Crangon spp., Carcinus maenas*), and bivalves (eg *Cardium* spp)

The alive adult organisms can be sampled (eg fish and shrimp, in rocky or mud ponds) or buy in local market (eg bivalves). All the specimens should be submitting to cumulative stress

conditions in aquaria during 3-4 days, eg hypoxia or starvation. Organisms will be frozen or preferably placed in liquid nitrogen immediately after collection.

a) Laboratory analysis

Fish, shrimp and bivalve specimens will observed, after defrost, using a dissecting microscope sex identified, total length measure, wet and dry weight determined. For all organisms macro structure maturation staged should be registered.
Condition factor will be determined based on formula $K = W/L^3$, where W is the body mass (mg) and L is the standard length (mm).
RNA and DNA contents can be analyzed according to fluorometric methods described by Esteves *et al.* (2000). Nucleic acids are extracted from a portion of 200 µg tissue in white muscle samples by adding 150 µl of 1% sarcosine and crushing the samples in ice (**Figure 1**).
After shaking and centrifugating, the samples are diluted to a final concentration of 0.1% using ice-cold Tris buffer. Fluorescence are measured photometrically using ethidium bromide. The amount of fluorescence originating from RNA (mainly ribosomal RNA) are calculated as the difference between total fluorescence (RNA and DNA) and fluorescence after ribonuclease A (type II-A) treatment, which are assumed to be derived from DNA. Fluorescence are determined by excitation at 365 nm and detection at 590 nm using spectrofluorometer (**Photo 1**). Sample concentrations of nucleic acids will determined from standard curves constructed daily using lambda DNA and ribosomal RNA of known concentration and of the appropriate range.

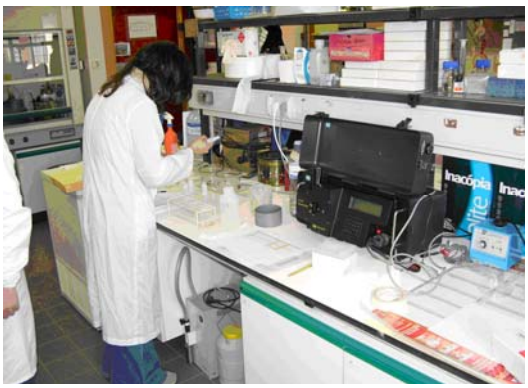

Photo 1. *Fluorescence determination with use of spectrofluorometer.*

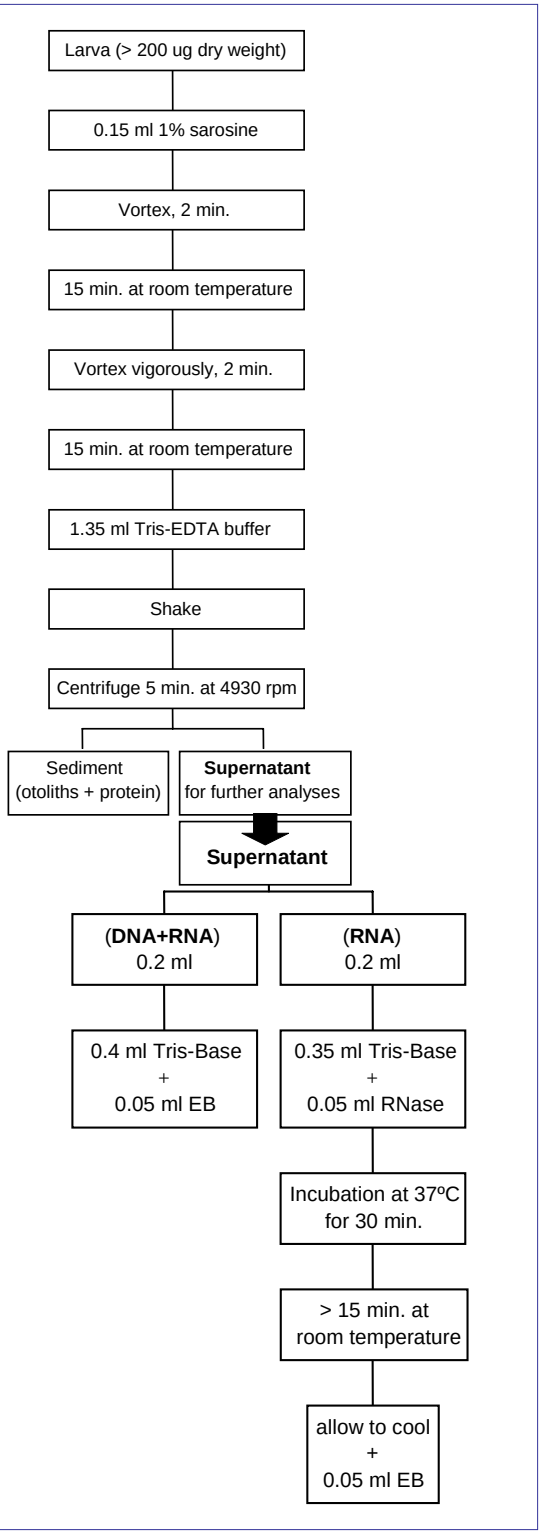

Figure 1. *Flow-chart of sarcosine extraction procedure and quantification methodology of nucleic acids described by Esteves et al. (2000).*

3. Materials and equipment

a) Field collection
You will need materials and equipment to collect water, fish, shrimps and bivalves:

- Baskets and containers for water collection and transport to the lab;
- Small hand-dredge for bivalve, crustacean and fish sampling in ponds;
- Clothing: water proof boots to walk on the margins, water proof jacket.

b) Laboratory experiments

- Calliper;
- Dissecting microscope and microscope;
- Owen and muffle for determination of organisms AFDW (ash free dry weight);
- Fluorometer;
- Centrifuge;
- Water bath;
- Automatic pipettes.

c) Data analysis

- Computer;
- Data sheet organizer.

4. Organizing the data

Basic statistical analysis
It will be necessary for morphometric condition index to organize the data according to the example in **Table 1**.

Table 1. *Morphometric index determined in Carcinus maenas*

Wet weigth (mg)	Total length (mm)	Sex	Weigth/Length^3
2120	16,72	Female	0,45
3580	19,37	Female	0,49
4050	19,48	Female	0,55
4510	20,84	Female	0,50
5780	22,38	Female	0,52
6050	24,93	Female	0,39
6570	23,52	Female	0,50
6880	23,97	Female	0,50
7350	25,72	Female	0,43
3630	19,86	Male	0,46
3650	19,87	Male	0,47
6020	23,63	Male	0,46
8700	26,27	Male	0,48
10450	29,4	Male	0,41
11990	28,36	Male	0,53
13600	31,03	Male	0,46
17700	31,72	Male	0,55

It will be necessary to perform a regression analysis between fluorescence units and acids nucleic concentrations (*see* **Figure 2**).

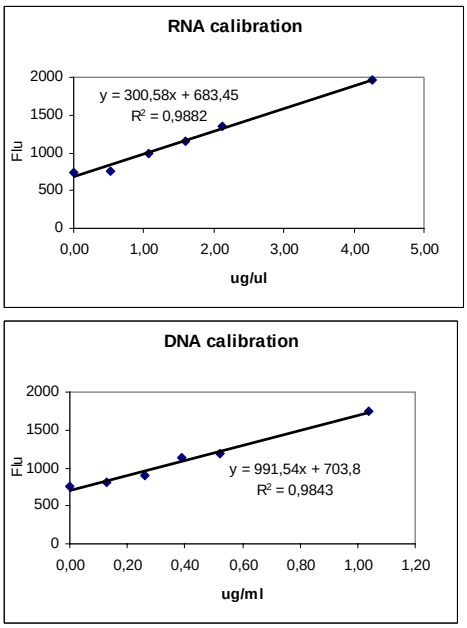

Figure 2. *Relationship between standard nucleic acids concentration and relative fluorescence readings.*

To analyse sex differences in condition indices it will be necessary to perform T- student test ($p<0,05$), see examples of results in **Table 2** and **Figure 3** and **Figure 4**.

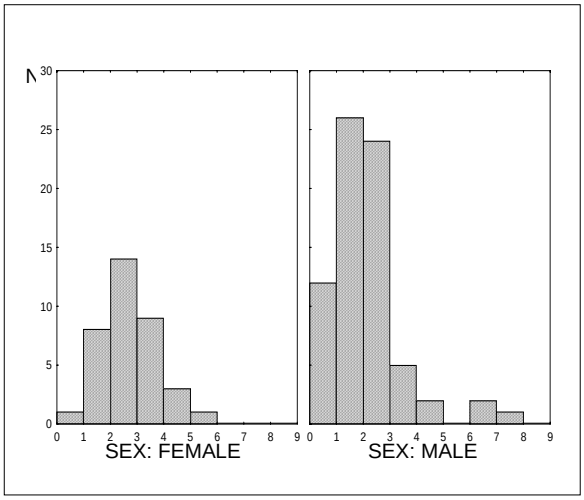

Figure 3. *Results of RNA/DNA ratios between males and females goby fish (Chicharo et al. 2007).*

Table 2. *Total length (mm), Dry weight(g) (for bivalves only meat weight and nucleic acid index of three species (mean ± standard deviation) and P values for differences between males and females of each species (P ♂♀) (Chicharo et al. 2007).*

Species	*n*	LENGTH	*P*	DRY WEIGHT	*P*	RNA/DNA	*P*
Pomatoschistus microps	126	45.41 ± 8.28	0.052	0.086±0.174	0.004	2.25 ± 1.27	0.006
Female	42	43.38 ± 8.96		0.086±0.141		2.68 ± 1.05	
Male	84	45.41 ± 7,78		0.081±0.19		2.03 ± 1.33	
Crangon crangon	155	29.25 ± 8.1	0.037	0.037± 0.013	0.01	8.06 ± 5.55	0.156
Female	135	29.75 ± 8.21		0.044±0 .014		8.3 ± 5.65	
Male	18	25.56 ± 4.71		0.035±0.012		6.28 ± 4.66	
Ruditapes decussates	38	33.4 ± 1,33	0.485	0.286±0.019	0.85	0.24 ± 0.257	0.009
Female	18	33.24 ± 1.51		0.285±0.012		0.33 ± 0.258	
Male	20	33.55 ± 1.16		0.29±0.01		0.16 ± 0.092	

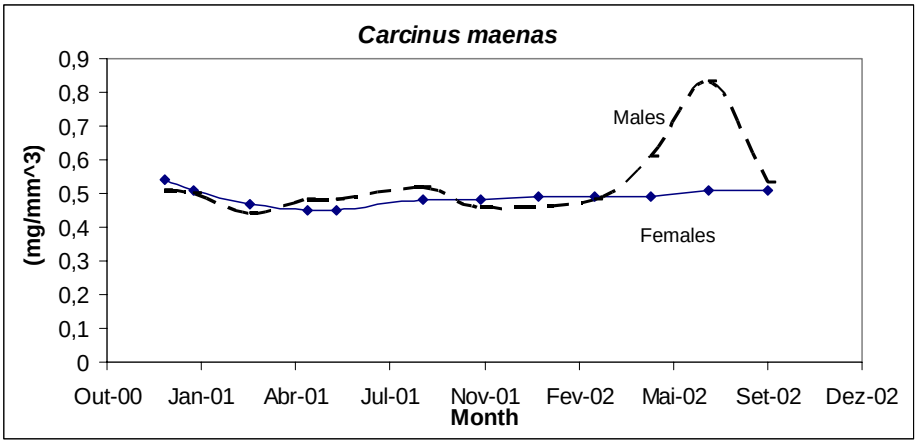

Figure 4. *Carcinus maenas variation of morphometric condition index between genders along different seasons.*

6. Analysing the results

To analyse the results try to have in consideration that your data may have been caused by sexual dimorphism, physiological or biochemical differences between sexes, or behavioral differences between sexes. Try to give especially attention to the investment of reproduction between the sexes of species analysed (Brokordt *et al.* 2003).

During the analysis try to answer to the following questions:

1. Which index is more sensitive to anthropogenic impact?
2. How did these ratios vary with the sex of the organism in different species?
3. Are males specimens more adequate to detected anthropogenic impacts?
4. If the frequencies of genders in samples are not representative of those in the population, what can happen to the analysis of population condition, eg. if females are over-represented?

7. Discussion

Discuss the obtained results with literature concerning the topic.

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