MATERNAL EFFECTS: FROM ENVIRONMENT THROUGH MOLECULAR AND INDIVIDUAL LEVEL, TOWARDS POPULATION ECOLOGY

RESULTS

The objective of the proposal was to evaluate the importance of natal habitat effects on the larval traits and the consequences for larval performance in the shore crab, *Carcinus maenas*. The general hypothesis is that larval performance is affected by salinity and temperature conditions of the natal habitats through maternal and embryonic effects on egg and larval traits. Egg and larval traits refer here to lipid profiling and expression of "stress-related" genes (mRNA of the Na^+-K^+-ATPase, Na^+-K^+-$2Cl^-$-cotransporter and hsp70 genes). *Performance* refers to survival (number of individual surviving the early larval stage) and duration of development to the second larval stage. There are two specific hypotheses: (1) Maternal effects and embryonic *salinity* and *temperature* co-determine the *egg/larval traits*; (2) larval *performance* is affected by *larval traits*. The *specific objectives* are to determine: (1) the effect of habitat on traits of early eggs, and how these combine with the temperature and salinity experienced by the embryos to affect the larval traits; (2) the effect of natal habitat on larval traits, and how these combine with the larval environmental conditions to affect larval performance.

3.1 Objective 1. Links: maternal effects-embryonic conditions-larval traits

Parasites as a component of habitat quality: Comparisons included females from two seasons (spring vs. autumn) and sites (Menai Strait and North Anglesey). In the Menai Strait, ca. 100 ovigerous female crabs were collected in the period May-July. The egg mass of most of these females were infected by a parasitic copepod (Fig. 1). Furthermore, by June-July it was clear that embryos from parasitized females did not complete their development. Parasite females can be seen consuming *Carcinus maenas* eggs and depositing their own eggs within the ovigerous mass. Two years of observation showed that parasites are frequent in spring-summer but only occasional in autumn. Further observation showed that, unlike *Carcinus maenas* embryos, parasites are sensitive to osmotic stress, so that it was possible to show that copepod parasites reduce the number of embryos developing successfully to advanced stages.

The data available on this parasitic interaction is scarce. The great majority of the literature on parasites of the shore crab does not list this group and there are only few publications (e.g. Gotto 1970) that refer to it as parasites of crustaceans and fish. Work done by the team previously in the German coast did not show any evidence of the presence of this parasitic copepod. Contacts with an expert taxonomist of the Senckenberg Institute of Germany led to a preliminary identification of this organism as *Choniosphaera maenadis* which is found in the Irish Sea parasitizing the gills of *Carcinus maenas* (Gotto 1970). This copepod appears to feed on embryos by siphoning material from them, while females lay eggs among the crab eggs.

From the ecological point of view the finding of a copepod parasite controlling the amount of larvae released by crab females is highly relevant for the work developed by the Fellow. If most egg masses are parasitized in the proportion found in the Menai Straits females, this copepod must have a strong control effect on crab recruitment. Spatial variations in the abundance of this parasite would therefore contribute to characterise the spatial variation in natal habitat conditions. This is in itself an important and unexpected result of the project and will motivate further studies.

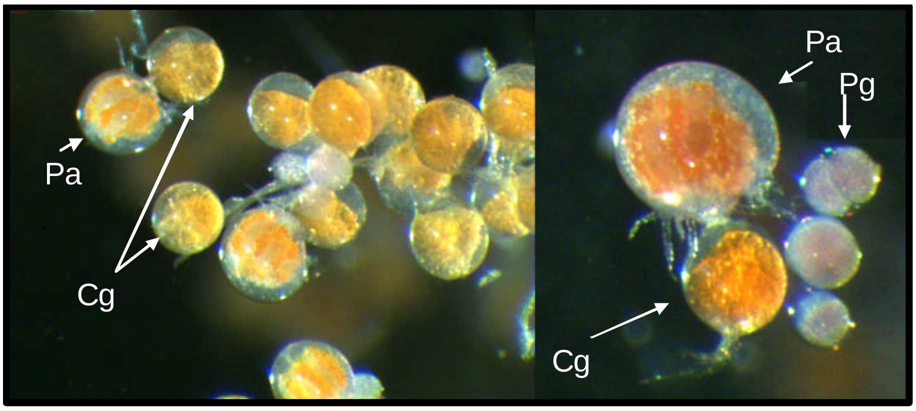

Figure 1. Parasitic copepods, probably *Choniosphaera maenadis*, in eggs masses of the crab *Carcinus maenas* collected in June 2009: (a) sample of an egg mass (b) parasitic copepod attaching eggs to the ovigerous hair supporting a crab egg. Symbols: Cg, crab eggs; Pa, adult parasite; Pg, parasite eggs.

Variations in larval body mass and elemental composition. Two years of data show that larval body mass in terms of carbon and nitrogen content vary with the season so that autumn females allocate more mass into eggs than spring females (Fig. 2 for year-1).

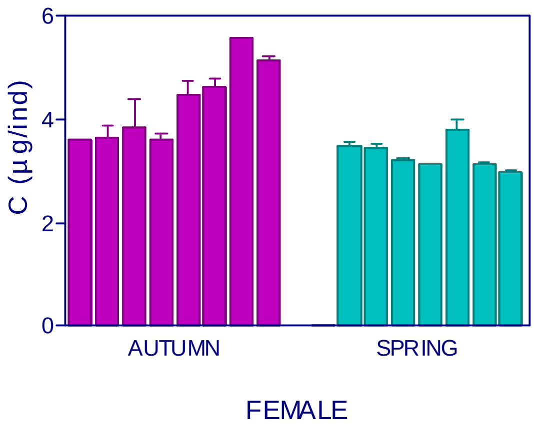

Figure 2. *Carcinus maenas*. Carbon content (expressed in µg per individual larva) of freshly hatched Zoea I from 7 ovigerous females collected in spring and 8 females collected in autumn.

Variations in lipid composition: In addition, there were important differences in the proportion of lipids and in lipid composition among seasons: summer larvae contain lower proportion of lipids (4-6%) than autumn larvae (7-11%). Larvae varied considerable in the proportion of free fatty acids (FFA), cholesterol and triacylglycerol (TAG), so that those hatched in autumn have higher levels of TAG and lower levels of FFA than those hatched in summer (Fig. 3). Neither salinity nor temperature experienced during embryonic development resulted in differences in lipid composition.

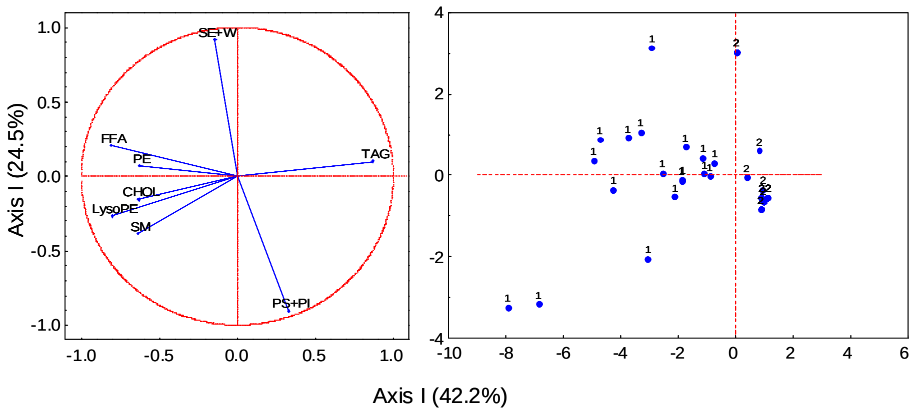

Figure 3. *Carcinus maenas*. Principal component analysis of lipid classes. The most important classes were triacylgycerols (TAG) and free fatty acids (FFA) which contributed to the formation of the first axis (left panel). Differences among larvae from autumn vs. summer occur mainly along the first axis and thus respond mainly to variations in TAG and FFA.

Variations in mRNA expression of genes involved in ion transport. Gene expression showed important differences among seasons and responded to embryonic conditions (Fig. 4). The mRNA expression of Na^+-K^+-ATPase was higher in autumn than in summer reflecting maternal influences, especially at higher temperatures and salinities: embryos laid in autumn responded to changes in temperature and salinity; gene expression in summer females was consistently low. The mRNA expression of Na^+-K^+-$2Cl^-$-cotransporter was consistently high in autumn but responded to temperature in summer: lower expression was found at low salinities.

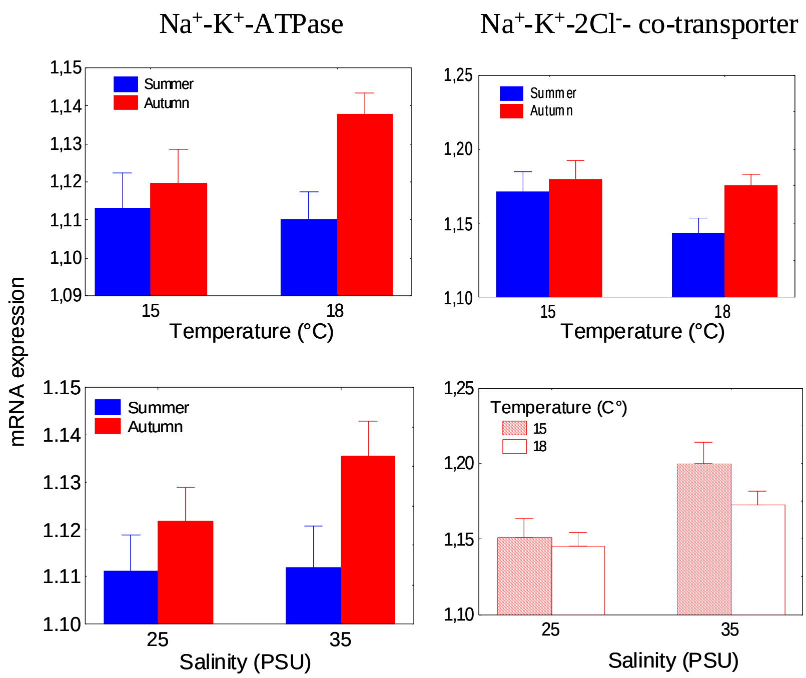

Figure 4. *Carcinus maenas*. Patterns of mRNA expression of Na^+-K^+-ATPase and Na^+-K^+-$2Cl^-$-cotransporter in response to salinity conditions during embryonic development in Zoea I larvae hatched in summer and in autumn.

In general, lower gene expressions at low salinity suggest that these low salinities had a negative effect. If gene expression responded to acclimation, we should have observed an increase in expression at lower salinities in order to compensate for loss of ions from the body. However, we observed the opposite pattern. This is consistent with the fact that the first zoeal stage of *Carcinus maenas* osmoconforms at this salinity.

3.2 Objective 2: links between larval traits and performance

Experiments to address this objective have been carried out satisfactorily. Crab females have been collected from two sites (Menai strait and North Anglesey coast) and seasons (spring and autumn) and maintained under two salinities (25 "estuarine" and 33 "marine") and two temperatures (15 and 18°C).

Tolerance to salinity (fig. 5): Larval salinity tolerance varied considerably among females (maternal effect component); this variation depended on temperature. Embryos reared at 12°C did not develop to viable larvae. By contrast, females reared at 18°C did produce healthy larvae. Freshly hatched Zoea I, from these females did not survive at low temperatures (12°C). Larvae exposed to low salinity (20) showed low survival, regardless of the female of origin (i.e. "estuarine" or "marine" females). When larvae were exposed to optimal salinity (33), there was a slight but significant difference in survival.

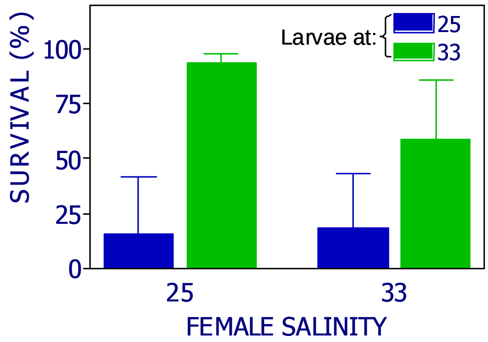

Figure 5. *Carcinus maenas*. Larval survival (percentage of individuals reaching Zoea II) when exposed to low (20) and optimal (33) salinity, obtained from egg bearing females exposed to estuarine conditions (salinity 25: left bars) and marine conditions (salinity 33: right bars). Data summarises survival of larvae obtained from egg masses of 14 females.

Maternal variation in carbon and larval survival: When freshly hatched larvae were exposed to 20, larval biomass did not play an important role on survival. However, when exposed to 33, larval survival was positively correlated with initial larvae carbon content (Fig. 6). This result suggests that an important component of the influence of maternal variation on survival is related to the lipids allocated by females to eggs.

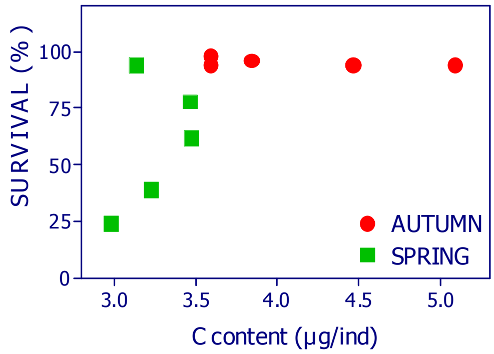

Figure 6. *Carcinus maenas*. Survival to Zoea II of freshly hatched Zoea I originated from autumn females (red dots) and spring (green squares). Each dot is the average survival of 50 larvae (reared in 5 groups of 10 individuals) produced by a female.