



CARCINUS NEWS

March 11 2010

Dear all in the CARCINUS PROJECT

This is the first of what will be regular (monthly) updates on the project more or less in the form of a newsletter. More detailed information will likely be put on a website when needed. This issue of CARCINUS NEWS will brief you on the project through 2009 and present some plans for 2010. If you have information about the project, I urge you to forward it to me so I can distribute it in this fashion to all of us. This excludes person to person mails or any urgent matters.

MEETING FEBRUARY 8

We had a meeting at the Department of Biology (BI) on February 8. Present were Jens Høeg, Henrik Glenner, Erik Hoffmann, Sten Munch-Petersen, Christoffer Grub and Tommy Kristensen. I (Jens) summarized the project work until now and some plans for 2009. Tommy and Christoffer reported on their work. Similarly, Sten and Erik informed us in detail about the work done by the DTU-aqua members.

MONTHLY SAMPLES: Throughout 2009 we from BI have visited DSC about every month. Unfortunately we missed some Summer samples. We should be very careful that this will not happen again in 2010! We normally have samples by bait from three localities: Venø Bredning, Løgstør Bredning and Lovns Bredning and at each place from stations at 1, 3 and 6 metres depth. We have surveyed the crabs so we can give the sex ration and size distribution of the various samples. This was done only on a subsample of the crabs from each station. But, , ALL sampled crabs were surveyed for sacculina carcini (Sc). This included male crabs that are judged to me "modified" (i.e., with a broader feminized abdomen effected by a still iunternal Sc). The Sc parasitized (sacculinized) crabs were measured, determined to sex and the status (stage of development) of the Sc noted.

Sacculina carcini categories: We have ended up with grouping the parasites into these categories:

Modified: Still internal parasites that have modified a male crab abdomen

Virgin: Small, whitish, recently emerged and globular parasites up to ca. 4mm across. These either have no males or have only very recently received males but not yet started to mature the ovary

Immature: Smaller parasites with a maturing ovary effecting a yellowish colour, but before release of the first brood of eggs into the mantle cavity. The early individuals in this category are easy to classify, but the later ones cannot truly reliably be separated from young ADULT parasites (see below).

Adult: parasites at or after release of the first brood of eggs into the mantle cavity (= brood chamber). This includes "older externae" that have become somewhat overgrown and brownish but are judged still to be capable of functional reproduction.

Old adults: Very old unhealthy externae including partially torn ones. These are mostly very black, slack or filled with dirt and in our experience incapable of any successful reproduction. [Jörgen, do we KNOW is the males in these are still functional or perhaps degenerating or even dead?]

Scars: Scars from lost externae.

If both scars and externae were found (or double-multiple infections) this was also noted.

RECOMMENDATIONS FOR 2010:

RECRUITMENT OF STUDENTS. We badly need manpower. Henrik is very occupied in Norway and so is Jens in Cph. What with Christoffer soon engaged in thesis writing and exam it is hard to find people that each and every month can go to DSC. We can hire students for the surveys but each visit needs an experienced supervisor. So both for this and other projects, we will again design a *recruitment poster* and display it at KU in various places. Each of you please be on the look out for students for speciale projects or bachelors project or short term projects.

Operating procedures monthly samples: Christoffer and I will write a sheet with SOP (Standars Operating procedures) for the DSC visits and crab surveyes. The reason is that the counting and tallying proceeded differently on some visits. This makes any Materials and Methods section (especially for Christoffer) a bloody nightmare so we HAVE to follow fixed procedures and be certain we stay at DSC long enough to follow them. Stay tuned for these simple rules.

Sacculina Categories: We should likely drop the separation into Virgin & Immature as this is rather difficult. I recommend that we retain only "immature" and "adult". As "immature" we classify ALL that clearly are BEFORE the first brood release (= virgins and those of the old category that are with certainty not yet adults). All other Sc become Adults. I will enter this into the "procedures" document.

Preservation of Sacculinized crabs: In the later 2009 samples we preserved all the "virgin" and "immature" parasites. This is top check on the presence of cyprid males and for some morphological examinations of the mode of male implantation.

Recommendation: I find it foolish, when we get all these parasites, not to preserve them all. I believe in 100% alcohol is best since this is useful for molecular purposes and (although less suited) for morphology. When needed, some special samples can be formalin preserved for morphology (such as perhaps all the virgins). I will order some suitable buckets that can be used. We should thus fix each category of sacculinized crabs from each station. With three localities and three depths this equals $3 \times 3 \times (\text{categories}) = 9 \times 6 = 56$ different samples per session. I suggest handling this at detailed below (appendix crab preservation).

Keeping of live crabs: We will ask DSC to keep some of the crabs alive for us. Specifically we will try:

- to ablate externae and see if and with what frequency and speed they regenerate new externae! WE have done some pilot experiments at BI (by Romualda) and it seems that, unexpectedly, regeneration does occur.

- to keep "virgin" externae. This will show by lab observation how long they can live on the crab and still remain attractive to male cyprids. We already know from Jørgen's extensive 1984 paper from the Isefjord that such virgins that fail to receive males will soon degenerate and die. But it would be interesting with a closer look and also resting them (Jens Höeg project) against male cyprids.

- Possibly keep "modified" male crabs alive and monitor if and when they develop virgin externae. That they do develop virgin externae is unquestionable (except where the classification as "modified" has failed). But this procedure will automatically also generate virgin parasites for observation (see above) and experiments. Such virgins can also be important in the behavioral experiments planned by Kim Mouritzen.

DTU-Aqua activities: Erik and Sten have repeatedly done quantified sampling of crabs and surveyed them for parasites (but not classified onto parasite categories as detailed as BI). They have also performed capture-recapture experiments. Perhaps Erik and Sten can mail me a brief of what and how they did this. Just a page or so for the next newsletter. Also, I would like from you an outlook for 2010: What have you planned to do. I know you gave some info on feb. 8 but we did not keep minutes of the meeting :-)

PAST AND FUTURE PROJECTS

MODIFIED CRABS (Tommy Kristensen): Tommy has in a student project been looking into the question of the modification of the male crabs. He briefed us on this on Feb. 8. Tommy's data is clearly publishable. Kim also by means of students has some data, that were acquired and analyzed somewhat differently. Still, the plan is to try and fuse all the information into a single paper to avoid duplication. We hope and urge that this materializes soon. Tommy will pass his project exam hopefully within the next two months.

CRAB AND PARASITE POPULATION STATISTICS (Christoffer Grub): This is the main stay of the BI efforts and a speciale (MSc) project on part of Christoffer. He will pass his exam later this year and so perhaps not be available for the project. Christoffer has until now generally been the "chief" at the DSC visits and the data is compiled with him. He is presently engaged in organizing the data in a spreadsheet etc. We hope also this will lead to one or several papers. But as yet we do not have an overview over the data. One thing is apparent. There are many more virgin/immature externae of *Sacculina* in the late autumn and early winter than expected. This was clear in the Nov. and Dec. samples. This is truly interesting and should be followed up (also by checking next the situation in 2010 and 2011). It touches on when and how many male larvae may be available into the Autumn or whether, unexpectedly, the virgins could stay healthy in the field for a long time and become implanted with males when they appear in the Spring. At present this is uncertain.

GENERAL PARASITE LOAD ON CARCINUS (Therese van Driel): Bachelor student Therese van Driel did a major survey of the total parasite load in *Carcinus* at all localities and depths. As expected, she found virtually no *Sacculina* at Lovns, but here a very, very high percentage (close to 100%) were heavily infested with one (or perhaps two) species of trematodes on the hepatopancreas. Therese notes prevalence but also "load", i.e., the number of trematodes in each crab. Her data on the isopod *Portunium* were also very interesting. I will place the whole Thesis at this website also (for the time being) to be used for other files until Henrik gets the real Website running!

www.bi.ku.dk/jthoeg/limfjord

We believe Therese's results warrants a publication. But we may need to rerun some of the statistical tests as she may not have been using the most appropriate ones (maybe Kim or Sten can assist?). We also need to first identify the trematodes to species. Henrik will look into this using DNA, but it may be necessary to resort to morphology (which requires some delicate manipulation to get the metacercariae out of their cysts). Therese is in Australia now, but may be able to assist long-distance.

Ditte collected a number of specimens of the gastropod hydrobia in December last year. Hydrobia is extremely common in Lovns Breeding and is a known intermediate host for many trematod species. It is therefore most likely that the hydrobia species found in Lovns is the preceding host for the trematod and therefore heavily infested with sporocysts. We surely needs to check that. Unfortunately, the Hydrobia samples got lost, but a new attempt will be made as soon as the weather allows it, right Ditte?

CRAB IMMUNE RESPONSE (Romualda Budvytyte): Romualda has been working with Henrik on taking blood samples and scoring blood cells numbers as an estimate of the crab immune response. This also involved longer term monitoring of crabs kept in the lab and, as one experiment parameter, the artificial removal of the externa. The data gathering has only stopped a couple of weeks ago and we will now look at the results. The goal is to monitor the effect of the parasite (in its various phases) on the host crab and the effect on the crab when the parasite is lost (and this obviously ties in with the possibility that a lost parasite may regenerate a new externa).

MOLECULAR WORK (Henrik Glenner and postdoc): Henrik and a new postdoc to start at Bergen will now commence the molecular work we have planned. WE shall detail on this later, but it will at least comprise the attempt to score the load of parasites using DNA (finding parasites with known DNA profiles) and microsatellite work to eventually characterize the crab and *Sacculina* populations. Cm satellites are in the literature and Henrik and Jens have developed *Sacculina carcini* microsatellites in a previous project suing crabs from the Limfjord area.

MODIFICATION OF CRAB BEHAVIOUR (Kim Mouritzen and student). In this project, lead by Kim and his specialestudent, Karen Beck, we shall look into the modification of crab behaviour by *Sacculina*. There are many aspects to this that can be pursued. We have agreed that up to Dkr 100,000 can be used for this part which we are certain will also result in one or several papers. In 1980-82 Lützen had a specialestudent that did a somewhat similar project. Unfortunately the report is not present any more in our files but should be available

from the Department files. At least, Lützen and I have some good suggestions as to what observation could be worth pursuing in addition and we have already had a meeting with Kim and the student on this.

SALINITY TOLERANCE OF SACCULINA LARVAE (Student wanted): This is an important part promised in the original project application. We surmise that the scarcity at Lovns of *sacculina* could be due to the low salinity. This offers the crabs a salinity "refuge" from Sc but this may be off set by mortality due to anoxia ? or the heavy load of trematodes found by Therese. At least we need carefully planned and physiologically relevant tests of nauplii and cyprids to various salinities and, perhaps, also temperature ranges. Salinity tolerance might well vary with temperature? There is next to no info on this although another *sacculina* species has recently been studied. WE desperately need a speciale student for this.

Appendix: Crab preservation: When sorting/recording out crabs from a particular locality-depth (= station) we throw the sacculinized into as many buckets as we have categories. We then put the crabs from each bucket into a plastic net with a prepared label stating the station. All such nets are put/fixed into a single bucket with the fixative (100% or 96% alcohol, or formalin when appropriate) labelled with locality and data. This produces three containers (each containing labelled nets) per survey and thus eases the storage/handling. nets can easily be retrieved again from the containers for further processing of samples when needed. If a particular sorting bucket happens to contain too many crabs for, we randomly put only every second or third into the fixation netbag and add a label stating that + record this in the logbook. The containers can hopefully be stored at DSC until needed.

PROJECT PARTNERS

Vip (*very important personel*)

Jens T. Høeg KU
Henrik Glenner UB
Jørgen Lützen KU
Erik Hoffmann DTU
Sten Munch-Petersen DTU
Kim Mouritzen AAU
Ditte Tørring DSC
Christoffer Schander UB

Students

Christoffer Grub KU
Tommy Kristensen KU
Romualda Budvytyte UB/U Klaipeda
Therese van Driel KU
Karen Beck AAU