THE COST OF SEXUAL PARASITISM

A project report to the Shackleton Scholarship Fund

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PARALOMIS GRANULOSA AND ITS ENIGMATIC FISH PARASITE

Understanding a living resource and its dependencies

Results from a research project
funded by the Shackleton Research Fund
October 2006

Background

King crabs (family Lithodidae) are amongst the largest arthropods on the planet, including an estimated 105 species that occur in cool waters from the intertidal depths down to the abyssal plains. Especially at higher latitudes, where productive continental shelves are covered with cold waters, king crabs support major fisheries. However, the once flourishing *Lithodes santolla* fishery on the South American shelf collapsed and today consists only of bycatch in the fishery for false king crab, *Paralomis granulosa*. This decline is paralleled by that of another economically important lithodid, *Paralithodes camtschaticus* in the north-eastern Pacific. Reasons for these declines include anthropogenic causes (over harvesting) and unexplained increased mortality due to parasites. The crashes of the two economically most important lithodid fisheries and the devastating effects on local economies demonstrate that a solid understanding of the interactions between the crabs and their environments (biotic and physical) are vitally important to develop a sustainable stock management strategy and ensure long-term protection of this living resource.

Sexual parasitism of crabs (carcinophily) by species of the snailfish genus *Careproctus* (Family Liparidae) that lay their eggs in the branchial cavities of crabs (family Lithodidae) is a well known phenomenon. Parasitized crabs apparently occur in only four genera (*Paralithodes, Lithodes, Lopholithodes,* and *Paralomis*). Carcinophily has been reported from the Sea of Okhotsk, Bering Sea, southeast Alaska and the northeast Pacific, Chile, South Africa and most recently, South Georgia and the Falkland Islands, where the host species parasitized are *Paralomis formosa* and *P. granulosa,* respectively. Although the eggs are clearly liparids (developing and hatching embryos and juveniles are easily identified to genus) the identities of the liparid species involved are generally unknown (op. cit.) because the eggs cannot be identified to species using morphological methods and adult fish have never been observed laying eggs.

Busy weeks in and around Port Stanley, Falkland Islands, October 2006

Aims of the study

Our aim was to study the sexual parasitism between *Paralomis granulosa* and the liparid fish involved. Our objectives at the beginning of the study were as follows:

1. How many species of liparids parasitize *P. granulosa* around the Falklands and are they identical to the ones known from South American inshore waters?
2. What liparid species are involved?
3. Does the liparid reproduce seasonally or continously?
4. Are there indications of tightly linked reproductive traits between host (crab) and parasite (fish)?
5. Does the parasitic egg clutch affect the survival of the host?
6. What is the incidence of parasitism in the *P. granulosa* population around the Falklands, and do the liparid (or other) parasites play a significant role in the ecology of the crab?
7. Are the observed interactions between individual liparids and crabs enough to explain the effect (if any) that parasitism has on the host population?

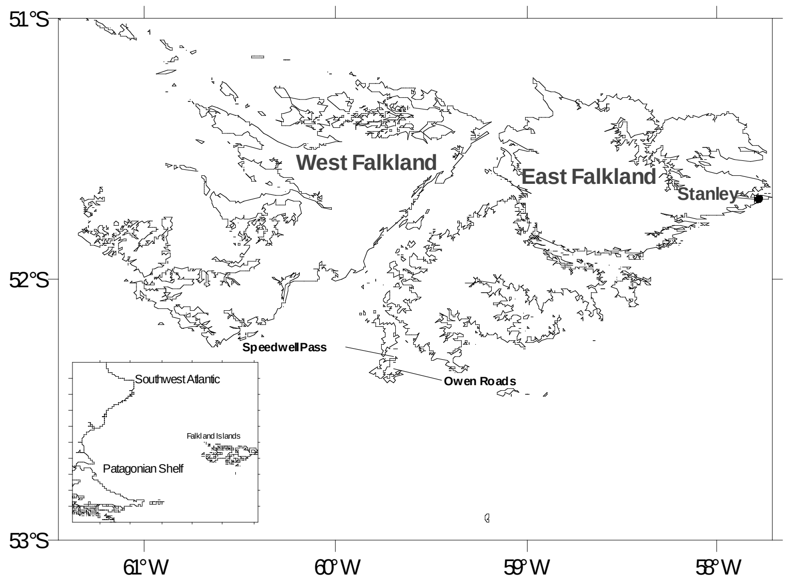
Careproctus falklandicus: Is this the species depositing the eggs inside the gill chamber of Paralomis granulosa?

Methods

Collection of samples

In October, we three spent our two October weeks intensively working on the problem. We sampled 563 crabs, of which 394 were males and 151 were females. Carapace widths ranged from 48-109 mm. Crabs sampled at Falkland Fresh seafood processors were almost all males because fishery regulations allow landings of only males of 75 mm carapace length or larger. Before the arrival of David and Christoph, Paul sampled 112 crabs on 19 and 26 September at Falkland Fresh seafood processors. On 17 and 23 October 2007, all three of us sampled 293 crabs at Falkland Fresh. They ranged from 75 to 109 mm carapace width and 71 crab had liparid egg clusters in their gill chambers.

On October 26-27th, the three of us worked on Chris May's boat, the "*Theo*", sampling female crabs and undersize individuals of both sexes.



Locations of sampling conducted on the "Theo"

We took a random sample of another 270 crabs, of which 26 were parasitized. In addition, another 44 female crabs were sampled non-randomly. During these collections, eggs in all stages of development were collected.

Method development

Lithodid crabs are heavily calcified crustaceans. Cracking their shells in order to identify parasitized specimens destroys valuable information about the positioning of the eggs inside the branchial chamber. Different methods for detecting infestations without having to kill the hosts (crabs) were tried: ultrasound, otoscopy (inserting an otoscope into the gill chamber and looking for egg masses), radiography, and candling (shining a bright light through the carapace from underneath). Candling provided the most reliable method.

Morphological and statistical studies

A total of 61 egg clusters were collected and fixed from infected crab. Eggs are very firmly glued together and although we treated them with an enzyme (Trypsin) to make it easier to separate them for counting, it did not help. Eggs from clutches taken from the crabs were counted, their diameters measured, and their development stages noted.

Development Stage	Code
Embryo - no obvious developemt	end
Tailed embryo - uneyed	tud
Embryo with small eyes	eys
Embryo with large eyes	eyl
hatching	hatg
hatched	hatd

Stage of embryo development.

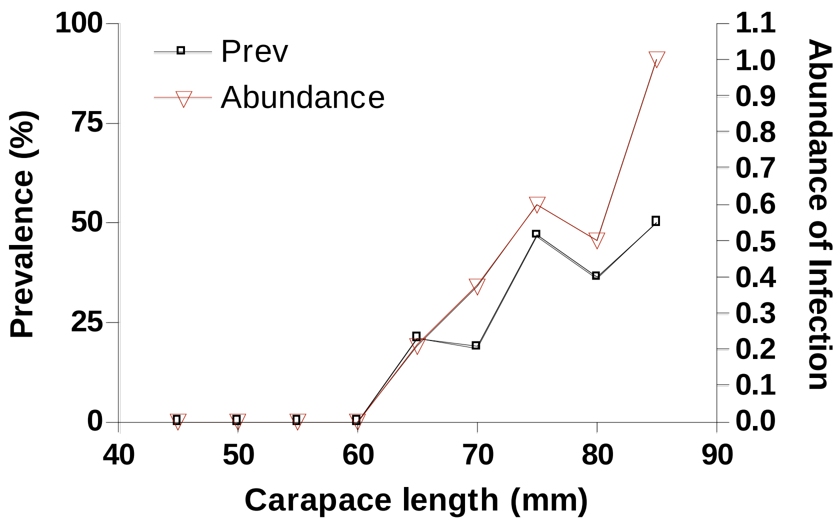
The following measurements were collected for each crab: carapace length (nearest mm), carapace width (mm), total mass (g), cheliped length and height (mm). We also noted the presence, number, position and in which branchial chamber the egg clusters were located, whether the individual was infected with a bopyrid isopod and in which chamber, whether or not it was infected with a rhizocephalan (*Briarosaccus callosus*), whether the individual was left handed and whether the females had a right or left handed abdomen. Simple linear regression and correlation analyses were used to examine the effect of cheliped height on the weight and number of eggs within infected crab.

Molecular work

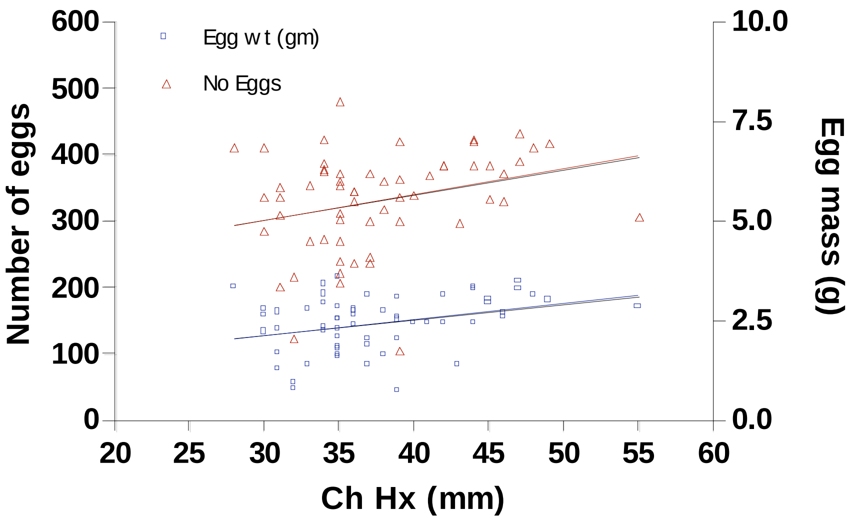
Genetic data have been collected to supplement morphological work in order to gain insight into the evolutionary history of crab association with liparid fish. For this, tissue samples of *Paralomis* crabs and their liparid egg clusters were fixed in ethanol and preserved for genetic analysis at the Alfred Wegener Institute's facilities. DNA sequencing of the 16S ribosomal RNA gene from the mitochondrial genome was carried out. This gene is known for its high mutation rate and has proven useful in studies focusing on molecular barcoding and genetic relatedness of species. The choice of the 16S ribosomal gene puts our study in a wider context in that it complements a study by Knudsen et al (2007) on the phylogeny of the family Liparidae, which lacks representatives from the Southern Ocean and the Southern Atlantic, however.

Results

The liparid species involved is probably *Careproctus falklandicus*, which has been collected in crab traps with crabs. However, because up until now we have been unable to obtain a specimen of *C. falklandicus* suitable for genetic analysis, we are unable to positively state that this is the species concerned. Egg number per cluster ranged from 104 to 795, and diameter from 2.05 to 3.03 mm, but most were about 2.5 mm. Our preliminary results show that the fish lay eggs only in males and the largest females and that more than one fish may lay eggs in a single crab. There was also a significant relationship between egg number and egg weight with claw size ($P<0.05$, $r = 0.29$ and $P<0.05$, $r = 0.33$, respectively). This may mean that larger fish prefer larger crabs, but further study is needed to validate this hypothesis. Twelve crabs had more than one egg mass. Initial statistical analysis indicated that there was no difference in weight (taking into account differences in size) between parasitized and unparasitized crabs. Furthermore, there appears to be a statistically significant preference among fish for laying eggs in the left gill cavity of crabs, rather than the right (χ^2 with Yate's correction: $P<0.05$, 5.02, df =1). We suggest that preference for one side over the other is related to crab morphology for some presently unknown reason.

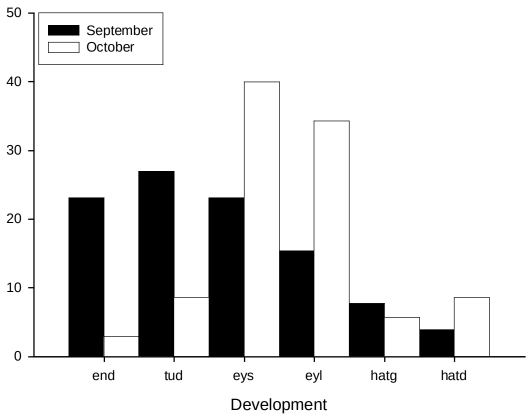


Relationship between crab size and the occurrence of snailfish egg clusters



Relationship between claw size and egg cluster number and weight.

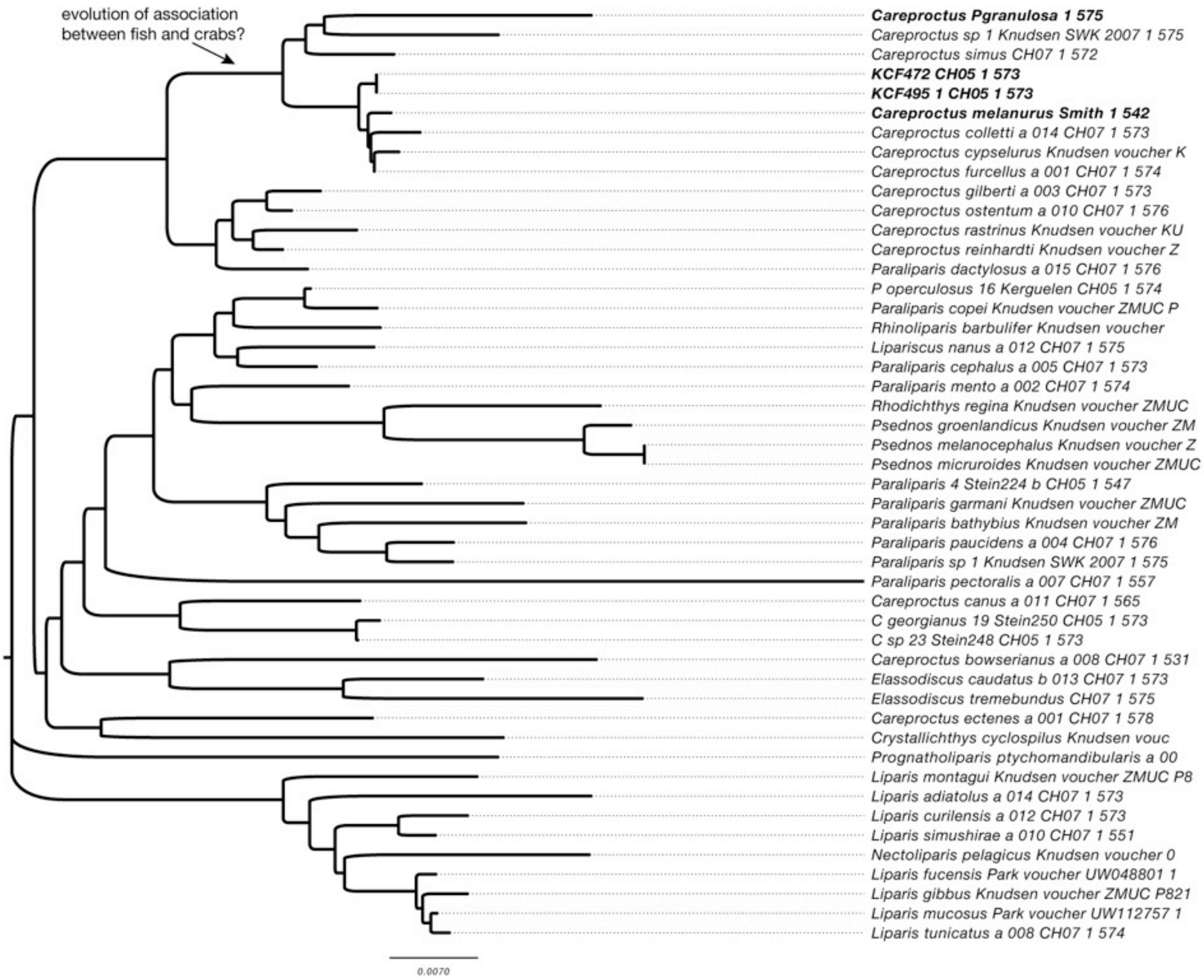
Interestingly, liparid embryos in crabs sampled in October 2006 were on the whole more developed than those sampled in September 2006. This needs further investigation and may suggest a peak in egg laying activity.



Frequency of liparid embryo development for September and October 2006.

Genetic relatedness of crab-loving liparid fish and others

The analysis of molecular data corroborates what has been established on morphological grounds already, namely that the eggs inside the gill chambers of *P. granulosa* around the Falkland Islands are liparid fish eggs. They further strongly suggest that the parasitic species belongs in the genus *Careproctus*, a genus that is also known for its carcinophyly elsewhere. Because we have been unable to collect a specimen of *C. falklandicus* suitable for genetic analysis, we have been unable to positively identify the species laying the eggs. However, we expect that it is *C. falklandicus* because that is the species that has been collected with crabs. The molecular tree established as a result of this study demonstrates quite surprisingly that crab-love may in fact be a major evolutionary novelty within the liparids. Irrespective of where the species occur, the liparid species that utilize crabs as a resource form a cluster of closely related species.



Molecular phylogenetic tree based on DNA sequence data from the 16S rRNA gene. The first species represents the liparid infesting Paralomis granulosa *around the Falklands. Species' names in bold print denote cases for which there is evidence of egg laying behaviour inside crabs.*

Unfortunately, Falkland Fresh has been unable to sell all the crabs Chris May has been landing, leading to reduction in frequency of landings from monthly to quarterly. This could affect our ability to study the life history of fishes and crabs in detail. However, there is interest among several other fishermen in the crab fishery, which may lead to more landings if a market can be found for the catch. Recent discussions with both parties suggest that the crab fishery will be starting again soon. This is a very exciting prospect because it means that samples will be easily available to us again.

There are many more questions to be answered, including the identity of the snailfish, how the fish identify crabs that are suitable, and how they time egg-laying to match the crab's molt cycle, and how long it takes the eggs to develop and hatch.

Conclusions

Based on our studies carried out during and after our stay in Port Stanley, October 2006, we can derive the following conclusions:

1. *Paralomis granulosa* around the Falkland Islands is parasitised by a fish belonging to the family Liparidae that deposits its eggs in the gill chamber of its host.
2. There is no molecular evidence for more than a single species of liparids depositing eggs inside the gill chambers of *P. granulosa* around the Falkland Islands.
3. The eggs hatch inside the gill chamber and early life stages stay associated with the crab.
4. The liparid belongs in the genus *Careproctus,* and is probably *C. falklandicus*. It is related to, but not identical with, the species known to infest other lithodid crabs in the Southern Ocean, however.
5. The evolution of crab association probably represents a major evolutionary novelty within the liparid fish, and to our knowledge is unique.

Future work

Our initial studies have helped clarify how the crab-snailfish relationship works and suggest many more interesting questions that we would like to answer. Based on morphological characters, the fish in question is probably *Careproctus falklandicus*, but we need to verify this conclusion through genetic analysis. Our results have prompted us to pursue the following questions in the future:

1. When do the liparids lay their eggs in the crab and what is their development time?

2. What are the effects on crab population health, growth, and survival? Are these related to number and size of eggs in each crab? Is there evidence that the crab population is affected by the liparids?

3. Is it likely that the crab fishery will affect the liparid population, and if so, what are the possible ecological effects of this?

4. Is the evolution of parasitic snail fish linked to the evolution of the crab family Lithodidae? Did crab and liparid species evolve together (e.g., coevolve)? Further study will help to answer this important question, which has implications for management of other lithodid species.

Acknowledgements

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