Massive encapsulation of larval *Anguillicoloides crassus* in the intestinal wall of the Japanese eel

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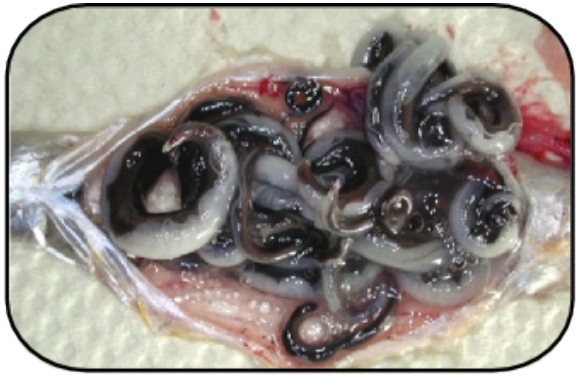
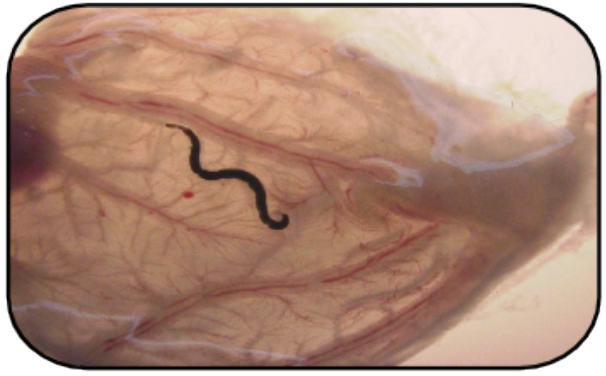
The study animal



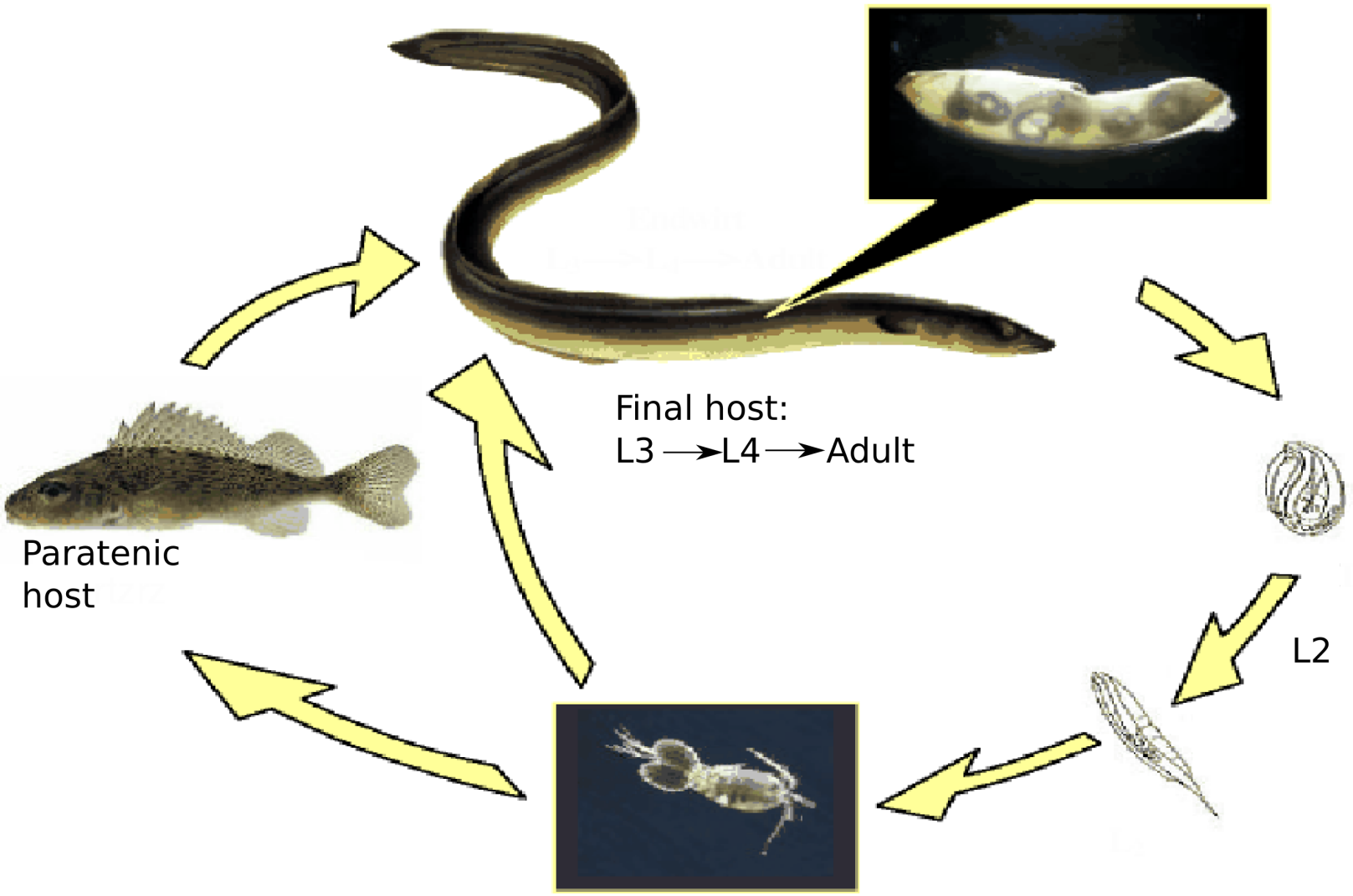
Parasites in the swimmbladder of the European eel



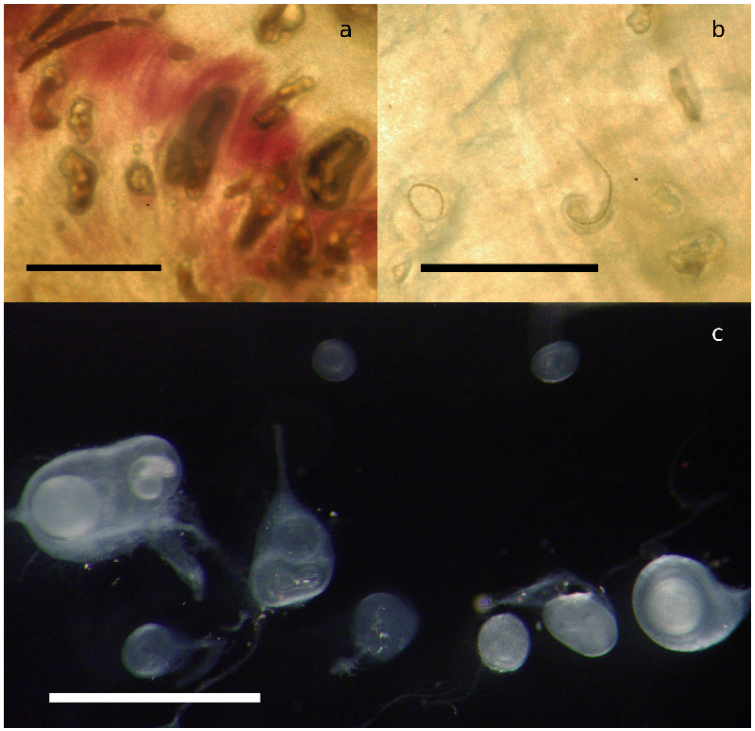
Parasites in the swimmbladder of the Japanese eel

- *Anguillicoloides crassus* was introduced from Taiwan to Europe in the early 80s
- In the novel host, the European eel, epidemiology differs dramatically
- Little is known about host-parasite interaction in the natural host

The live cycle of *A.crassus*



Initial observation



Encapsulated larvae of *A. crassus* from the swimbladder wall (sb. caps) and the intestinal wall (in. caps), scale indicates 1mm.

a Common observation in the swimbladder wall

b Common observation in the intestinal wall

c During preparation of intestinal capsules for PCR

Higher infective pressure in aquaculture

	n	prevalence (%)			mean abundance			mean intensity		
source		adult	larval	total	adult	larval	total	adult	larval	total
wild	40	32.5	2.5	32.5	0.6	0.05	0.65	1.85	2.0	2.0
±SD					±1.37	±0.32	±1.42	±1.91	±NA	±1.91
cultured	151	41.7	25.82	47.7	1.4	1.0	2.4	3.48	3.85	5.01
±SD					±3.32	±3.0	±5.0	±4.48	±4.88	±6.26
comparison p		0.38	0.003	0.12	0.17	0.001	0.027	0.15	NA	0.038

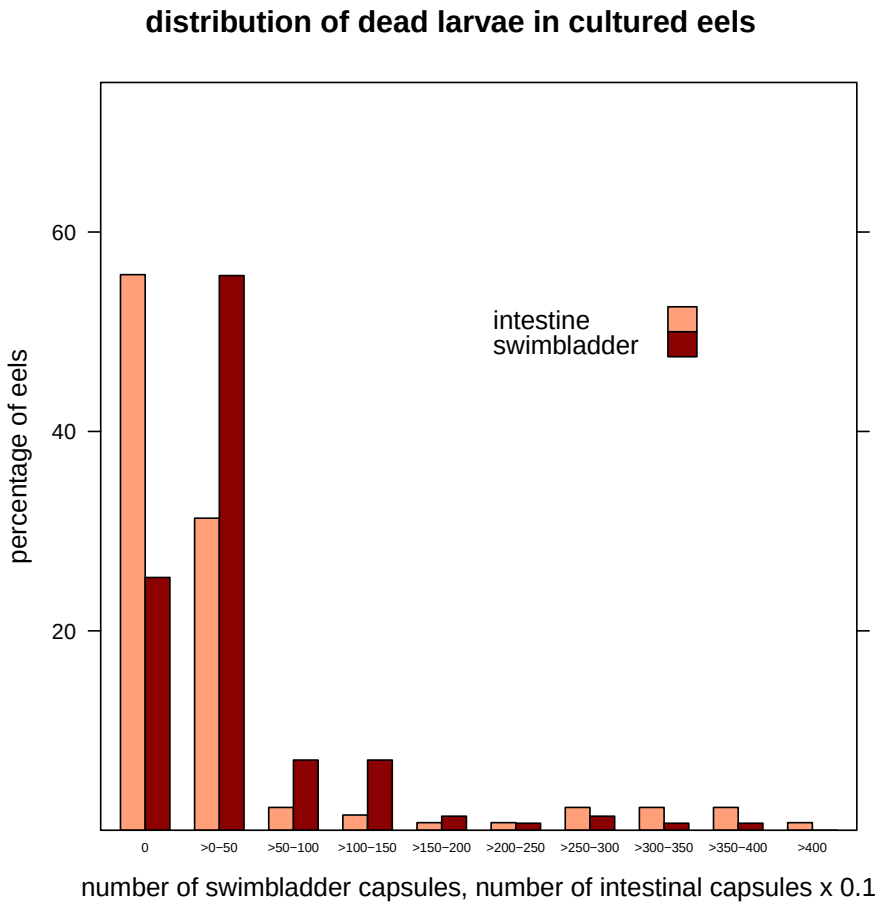
Infective pressure is higher in aquaculture than in the wild. This is only detectable in larval-infection parameters.

More larvae, more sb. caps, more int. caps

	prevalence (%)		mean abundance		mean intensity	
source	sb. caps.	int. caps.	sb. caps.	int. caps.	sb. caps.	int. caps.
wild	14.29	3.45	0.51	0.03	3.6	1.0
±SD			±1.74	±0.19	±3.44	±NA
cultured	74.65	44.27	34.57	346.01	46.31	781.64
±SD			±62.33	±942.74	±68.32	±1296.26
comparison	$p << 0.001$	$<< 0.001$	$<< 0.001$	$<< 0.001$	0.008	NA

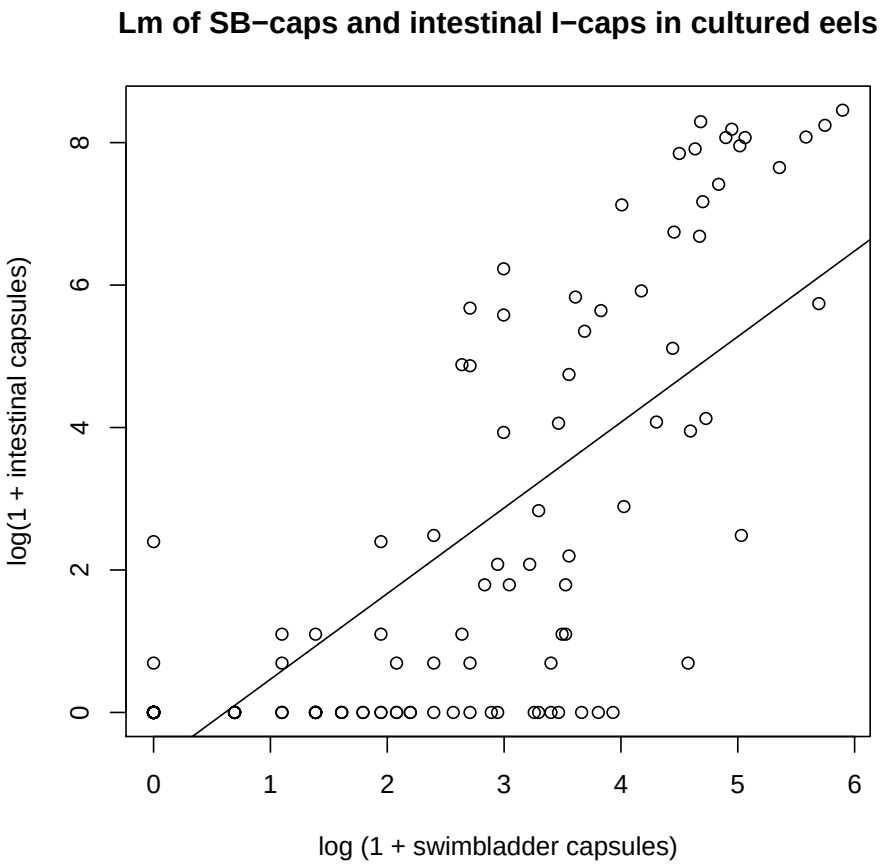
More sb. caps are found in eels from aquaculture. Int. caps are only found in aquaculture.

Overdispersed distribution of capsules



- Overdispersion indicates spatial aggregation
- Larval distributions are more overdispersed than adult
- sb. caps more overdispersed than larvae, int. caps more than sb. caps

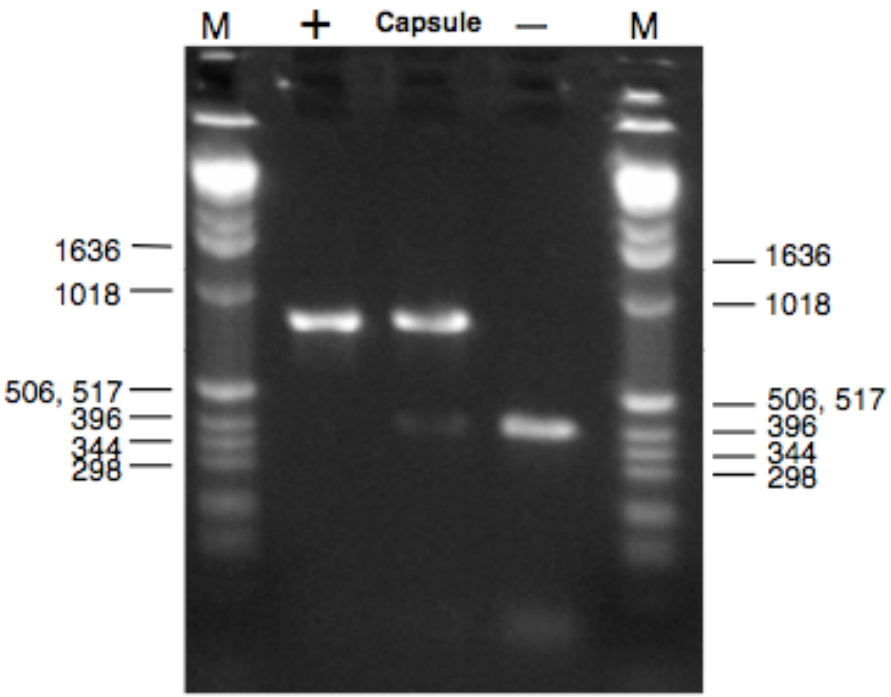
Linear model for capsules



The linear model on log-transformed data (to reduce heteroscedasticity):

- R^2=0.5914
- slope=11.53 on an untransformed scale
- Int. caps are never found in the abscense of sb. caps
- Relationship very hard to model (using other transformations or glms)

Amplification of D2-D3 loop of LSU



It is possible to amplify the D2-D3 loop of the large ribosomal subunit (LSU) of *A. crassus* when:

- Tissue is fixed in DMSO/salt (DESS)
- Capsules are opened prior to lysis

A distinct "nematode band" is visible on gel. Sequencing from this confirms the presence of *A. crassus*

Encapsulation can be reproduced in experimental infections

4 month after initial immunisation with 50 larvae Japanese eels were infected with:

Group 1: 2 x 50 larvae within 10 days (n=6)

Group 2: 2 x 100 larvae within 10 days (n=6)

Group 3: 2 x 300 larvae within 10 days (n=6)

Only in group 3 were larvae found encapsulated in the intestinal wall

Encapsulation can be reproduced in experimental infections

Comparison group	infection parameter	p	W
300:50	L3	0.863	16.5
300:100	L3	0.534	22.5
100:50	L3	0.510	13.5
300:50	L4	0.002	36
300:100	L4	0.002	36
100:50	L4	0.333	24.5
300:50	Adults	0.002	36
300:100	Adults	0.002	36
100:50	Adults	0.586	22
300:50	sb. caps.	0.119	28
300:100	sb. caps.	0.619	14.5
100:50	sb. caps.	0.164	27
300:50	int. caps.	0.002	36
300:100	int. caps.	0.002	36
100:50	int. caps.	1.000	18

Experimental infections differ from infections in the aquaculture

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Conclusions

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- Encapsulation in the intestinal wall occurs in Japanese eels at very high infective pressures
- Encapsulation in the intestinal wall follows encapsulation in the swimbladder wall
- Amplification of the LSU D2-D3 loop works even with a very high host contamination background
- Some aspects of experimental infection are different from semi-natural infections in aquaculture
- Studies on the intestinal wall of European eels (where infective pressure is high) are needed

miscellaneous

If you liked this talk about an unexpected observation during sampling in Taiwan, you might be interested in my second (main project) talk:
The transcriptome of *A. crassus* sampled by pyrosequencing
This afternoon in the Methods Section

Thank you for your attention and the following people and institutions for support

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lab use Mark Blaxter