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Laureata in Biologia Ambientale
all'Università di Ferrara

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Laurea Specialistica in Biologia Marina
all'Università di Trieste

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Dottorato di ricerca in Monitoraggio dell'Alterazione Ambientale
all'Università di Trieste

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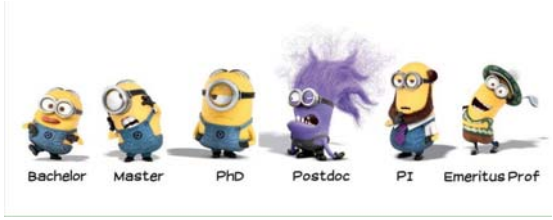
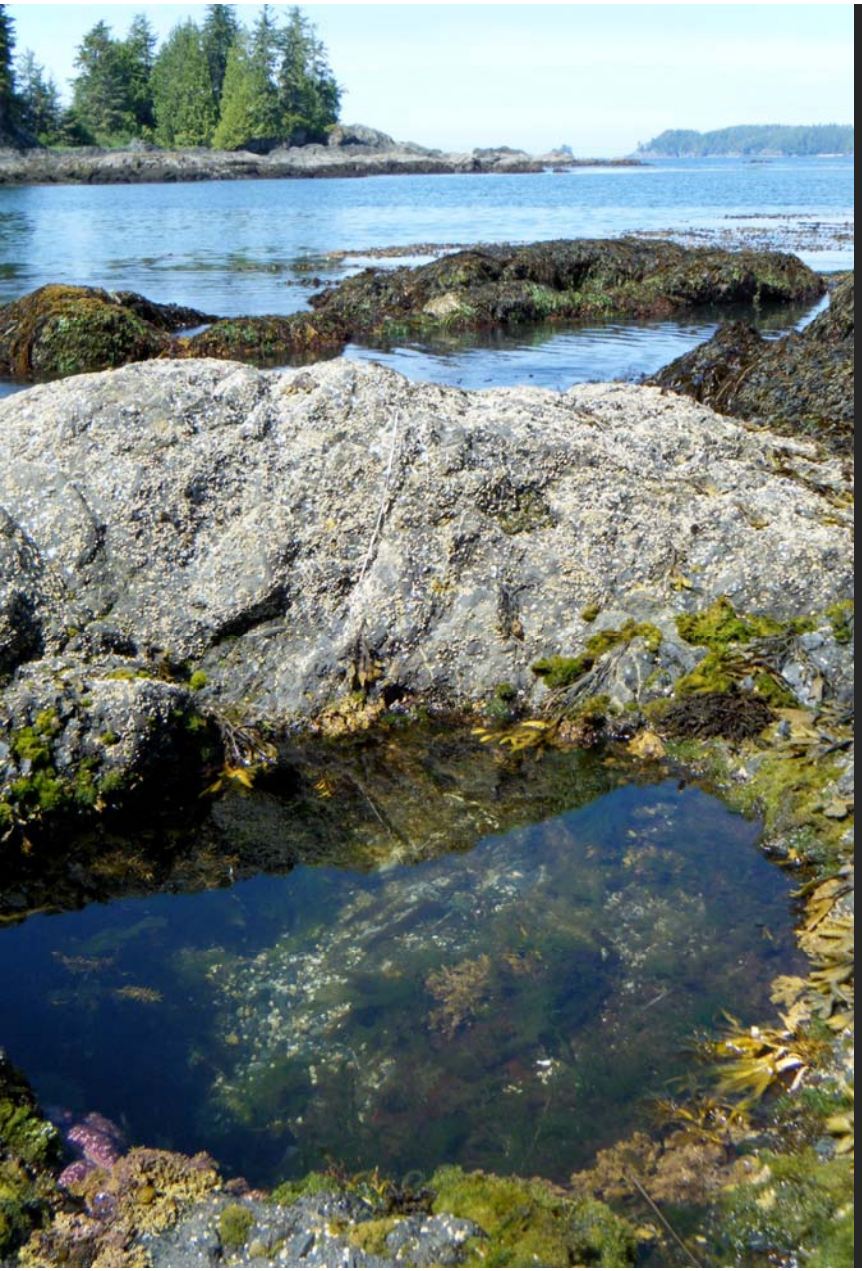
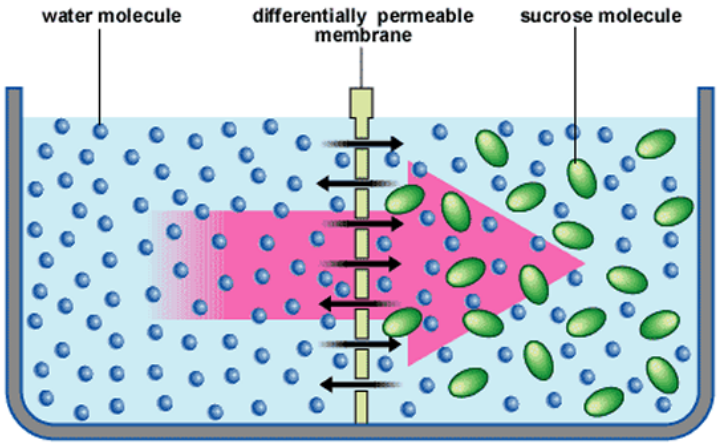


Collaboratrice in Immunologia, endocrinologia e monitoraggio
delle specie acquatiche
all'Università di Trieste

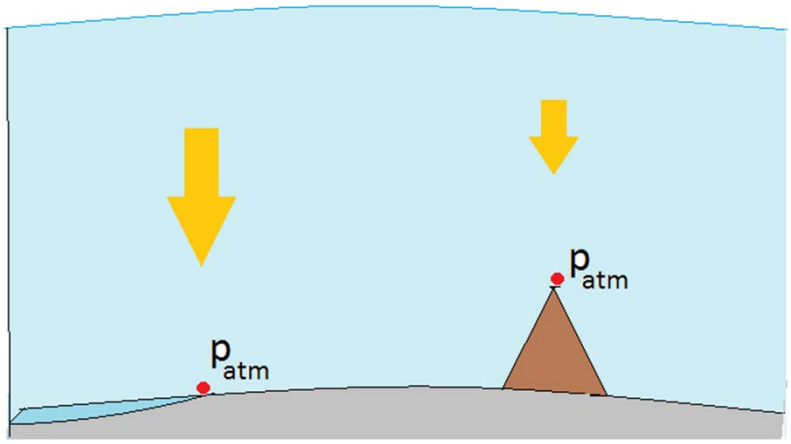


Special aquatic habitats

ADATTAMENTO AGLI AMBIENTI ESTREMI



Salinità

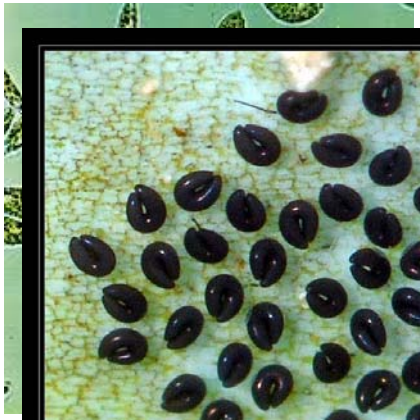


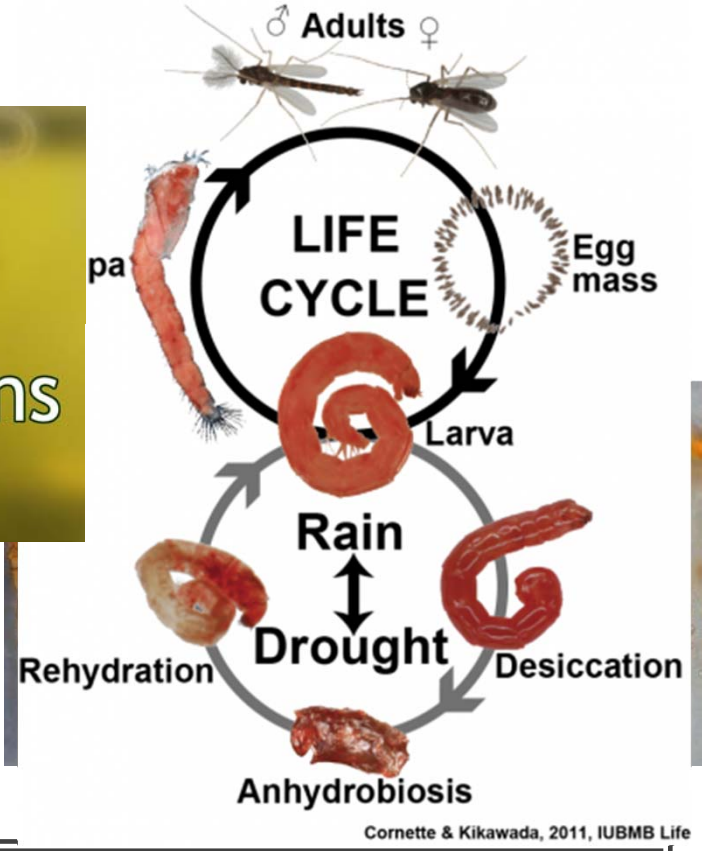
Pressione

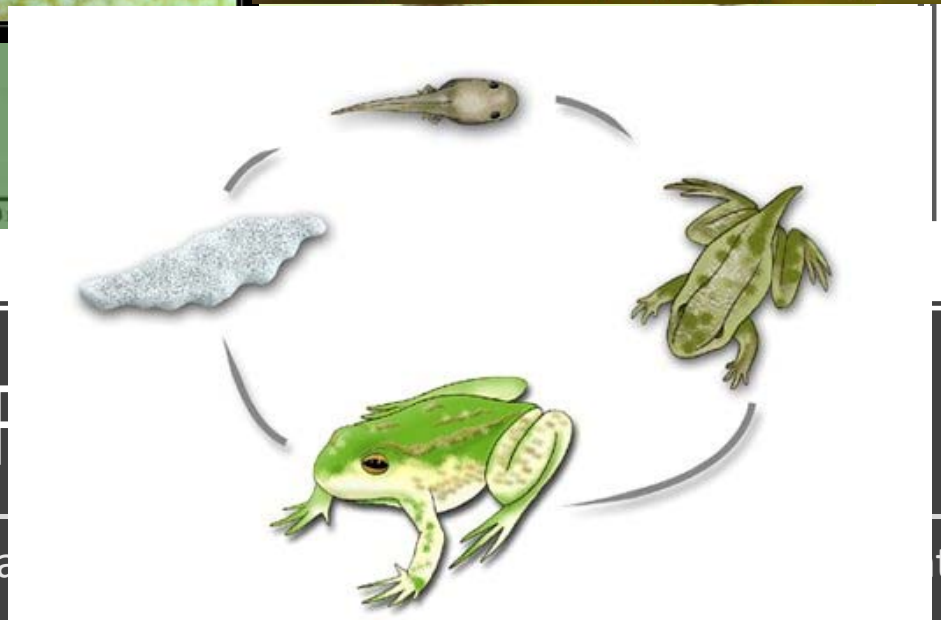
Temperatura

CORPI D'ACQUA TRANSIENTI

Sono, ad es., habitat dove la presenza d'acqua dolce è intermittente (pozzanghere, pozze, interstizi, substrati muschiosi, fessure nelle rocce, acqua raccolta nella vegetazione o nelle carcasse degli animali.





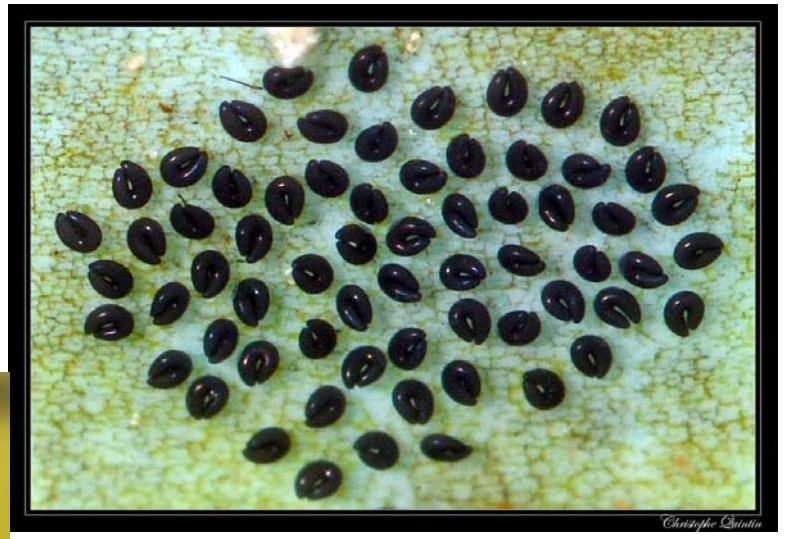


Ptychadena – tadpole densities up to 1000 m−2 of water surface

COR SIENTI

POZZE: a to drenaggio

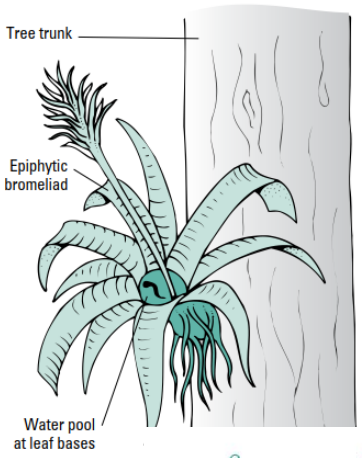
Pozze

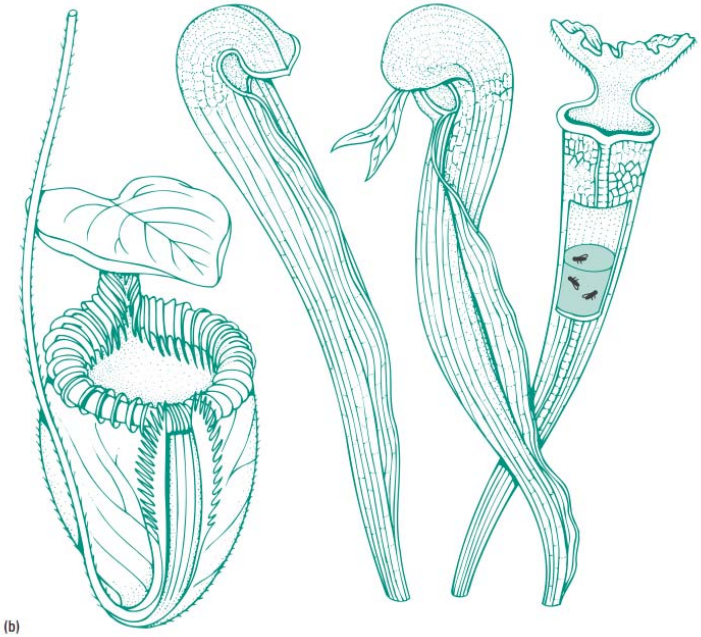


Puddle specialists must be relatively eurythermal and also tolerant of anoxia, but we have little detailed knowledge of their physiological adaptations

POZZE D'ACQUA IN PIANTE ED ANIMALI

Where such habitats occur in living plants they may be **well oxygenated by day**, but **have periods with high levels of carbon dioxide and low pH**; in animals or plant remains the water is commonly **strongly eutrophicated and may be anoxic**.



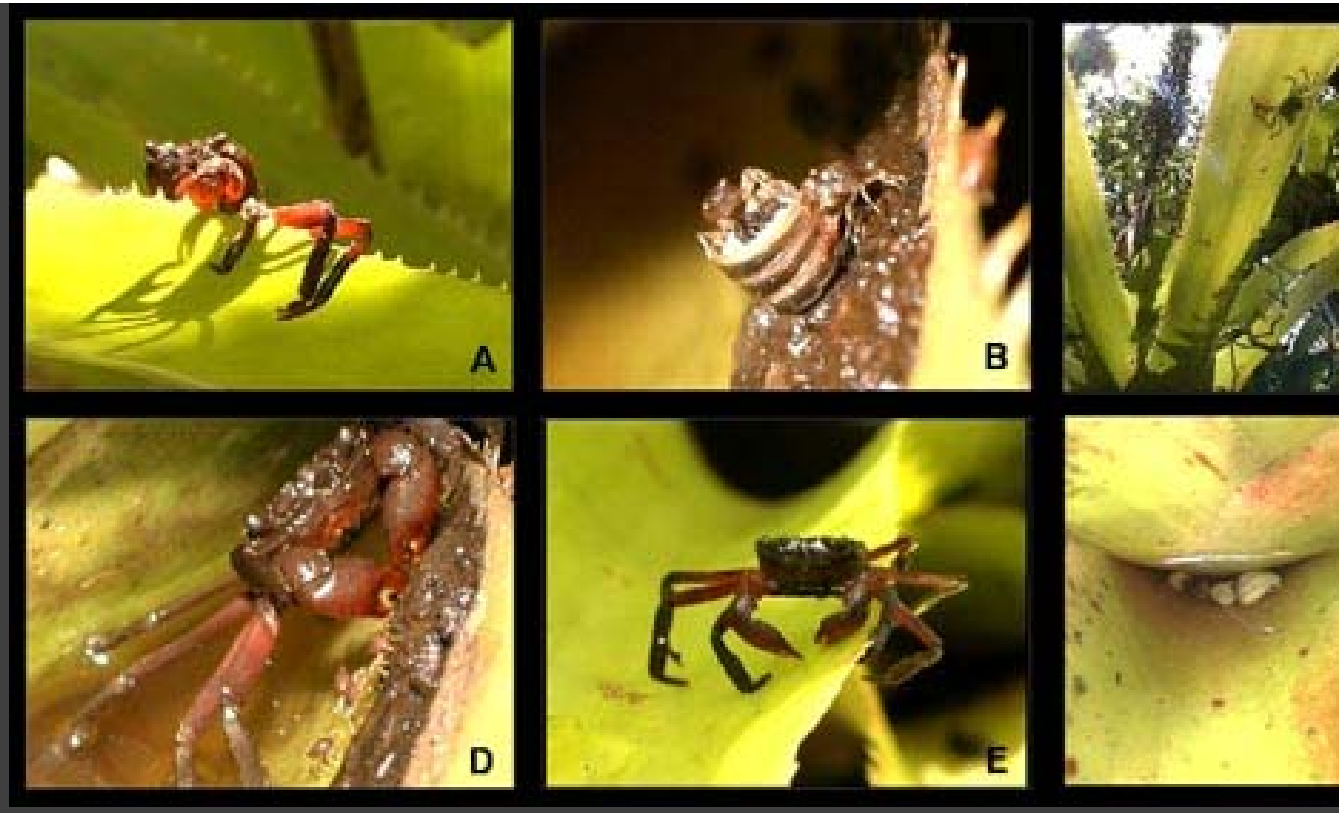


In alcune specie vi è la presenza di calcificazioni craniali che utilizzano per sigillare l'entrata delle loro tane.

Queste placche permettono ↓ tasso evaporativo dell'acqua (Evaporative Water Loss, EWL)

Quando le rane entrano nelle tane riescono a resistere al disseccamento

Aechmea paniculigera: the habitat of *Metopaulias depressus*



Metopaulias sp. The larval stages are completed in the low pH water of the tanks (usually pH < 5–6), but not in normal river water from the same habitat (pH 8). Recent evidence indicates that the crabs can achieve some degree of pH and Ca2+ control by importing fragments of calcareous snail shells into the tanks.

Vive tra le mangrovie e non necessita di tornare all’ambiente marino nemmeno per la riproduzione.

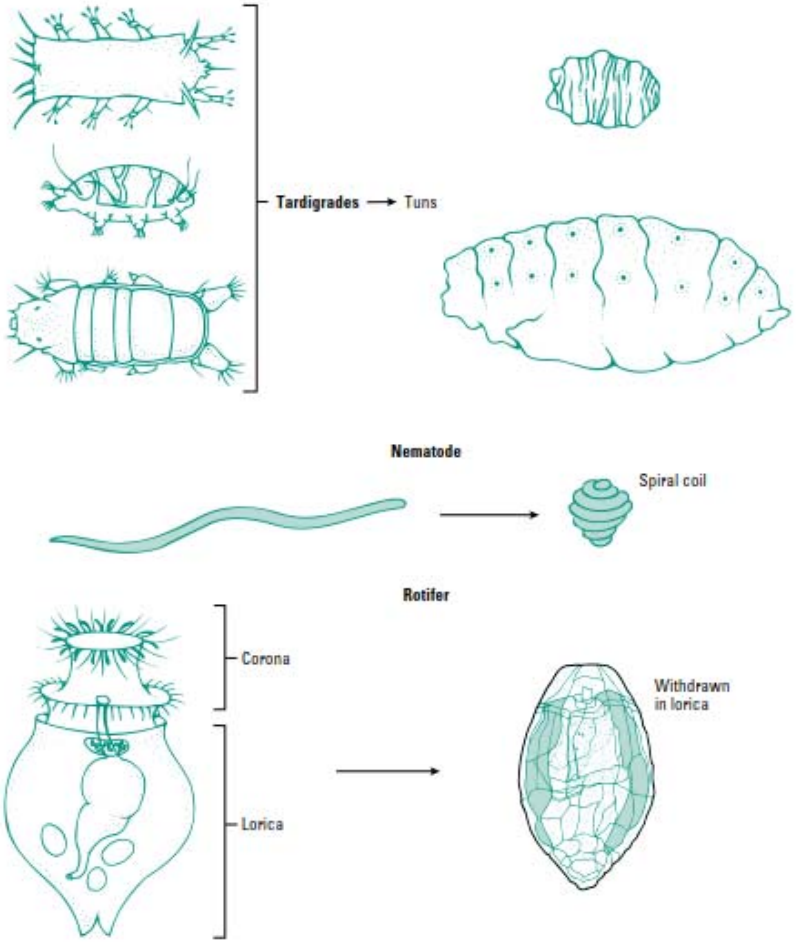
Sesarma sp

La ♀ trasporta le uova fino a quando trova un guscio di lumaca della taglia giusta--- lo riempie di circa 5 mL di acqua attraverso una zona pelosa della cuticola (capillarità).

I giovanili si sviluppano nel guscio monitorati dalla ♀ per circa 3 mesi.

Transitorietà estrema

Fauna that use cryptobiosis as a survival strategy in transient aquatic habitats.



Quando sono attivi, i tardigradi sono ricoperti da uno strato d'acqua ricco d'O_2

Quando l'ambiente si secca, essi vanno incontro ad una completa essiccazione.

In alcuni gruppi questa strategia avviene nei giovanili, nelle uova o nelle cisti, ma in altri (rotiferi, nematodi e protisti) anche negli adulti.

Cryptobiosis

Ametabolic state of life entered by an organism in response to adverse environmental conditions such as desiccation, freezing, and oxygen deficiency.

Anhydrobiosis

Organisms dessication tollerant

Ressurection plant

anhydrobiotic organisms can survive for **decades**, even **centuries**, in the dry state.

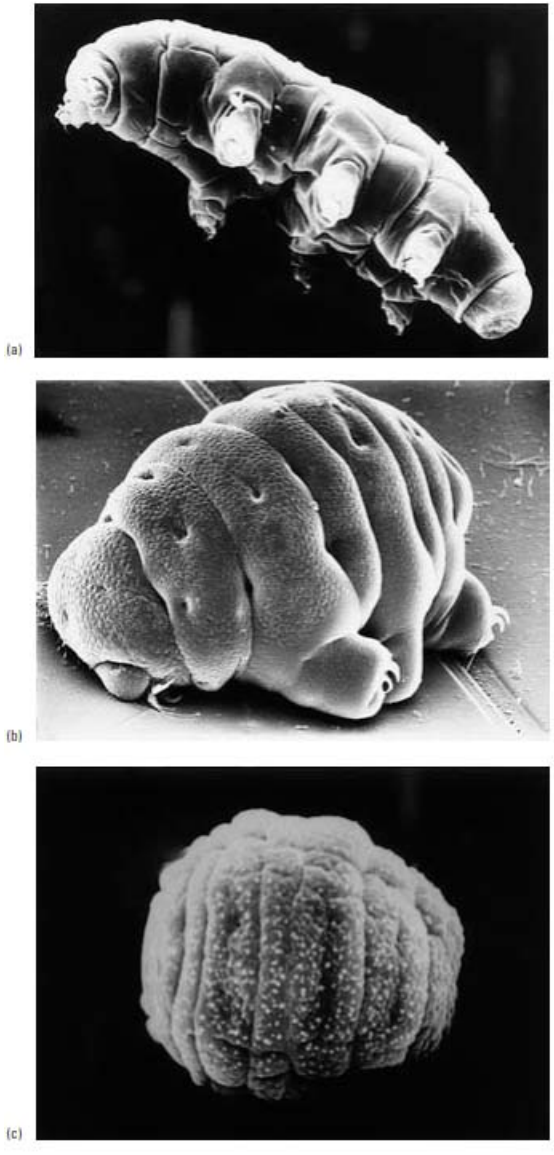
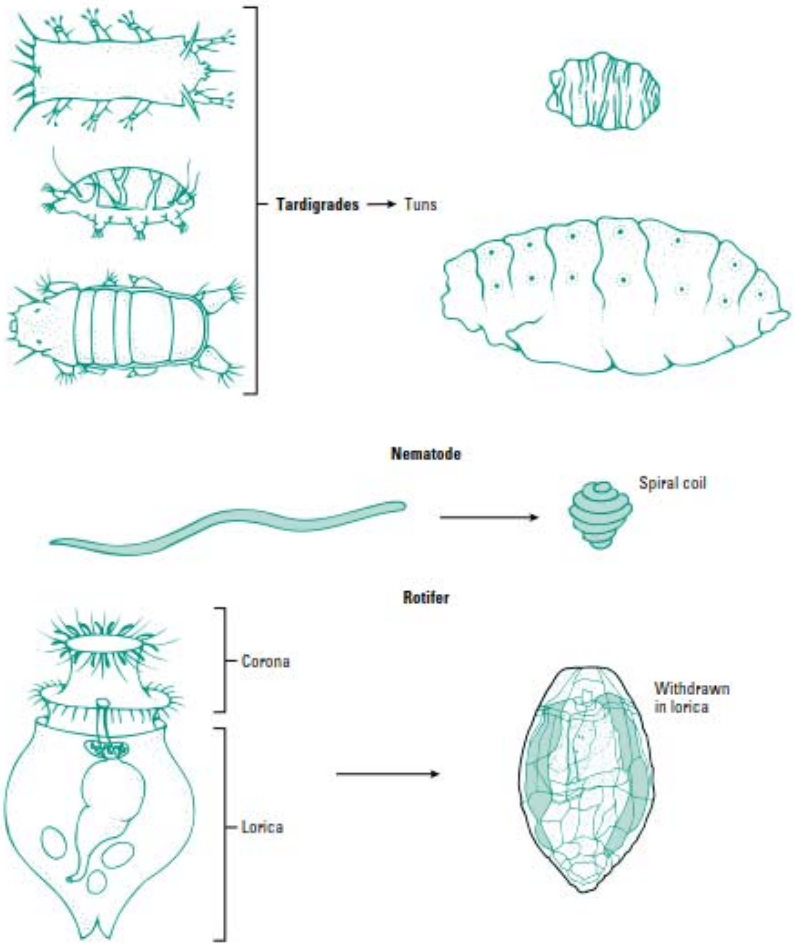


Fig. 14.3 SEMs of a tardigrade (a) in its normal hydrated state, (b) shortly after beginning entry to the cryptobiotic state, and (c) when fully into this state (the "tun"). (Courtesy of J.C. Wright.)

1. **Cambiando la morfologia** della superficie, inserendo di più aree permeabili della cuticola e modifica dello spessore relativo o chimica degli strati della cuticola.

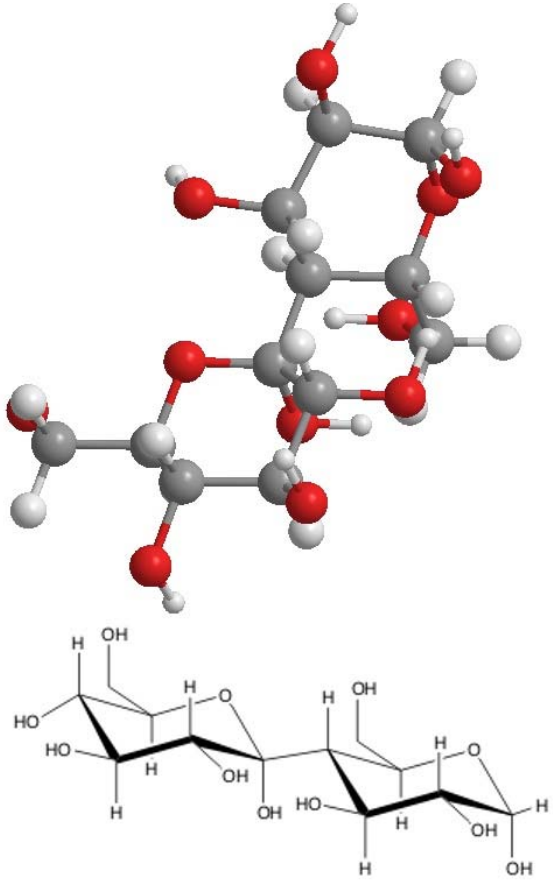
2. **Riducendo la permeabilità della cuticola**, con cambiamenti di fase lipidica e / o la produzione di cera, assicurando così che una quantità minima di l'acqua viene trattenuta

3. **Produzione metabolica** di una grande quantità di zuccheri, come il **trealosio** e il **glicerolo**, che proteggono i fosfolipidi e le proteine da danni durante la siccità legandosi alle membrane al posto dell'acqua per stabilizzarne le strutture. Questo processo è accompagnato dalla "**vitrificazione citoplasmatica**", che modifica il liquido intracellulare in un materiale simile al vetro.



La sintesi del Trealosio è catalizzata da 2 enzimi **T6P-sintase** e **T6P-fosfatase**.

La sua degradazione è controllata dall'enzima **trealase**



Le principali azioni:

- Interazione con e diretta protezione dei lipidi presenti nelle membrane e delle proteine (H lega i gruppi PO4 e altri residui polari.
- partecipa alla formazione «vetrosa» del citoplasma che deve essere mantenuta durante tutto il periodo di criptobiosi

Es. nei nematodi avviene in tutti i tessuti, ma principalmente nei tessuti muscolari e negli organi riproduttori.

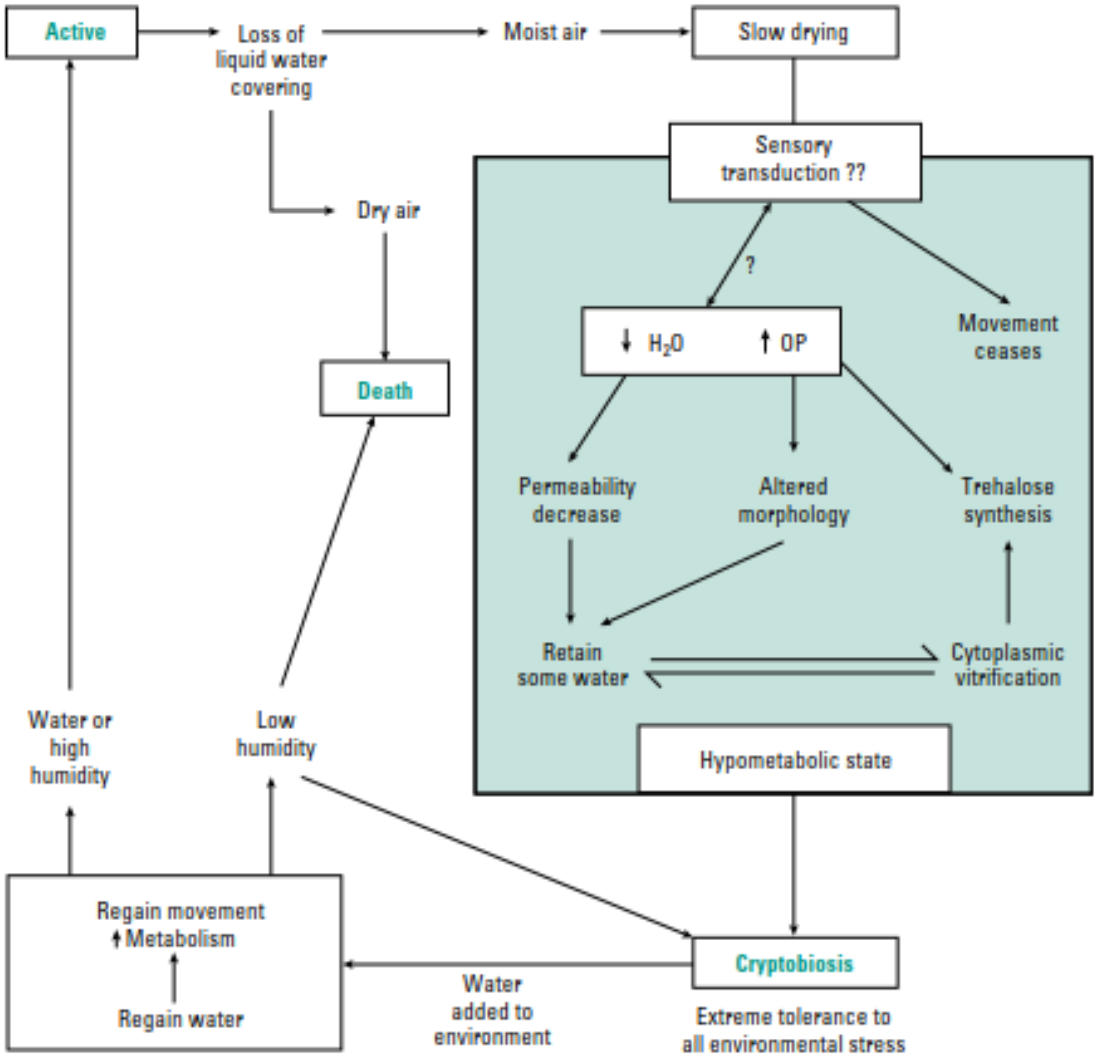
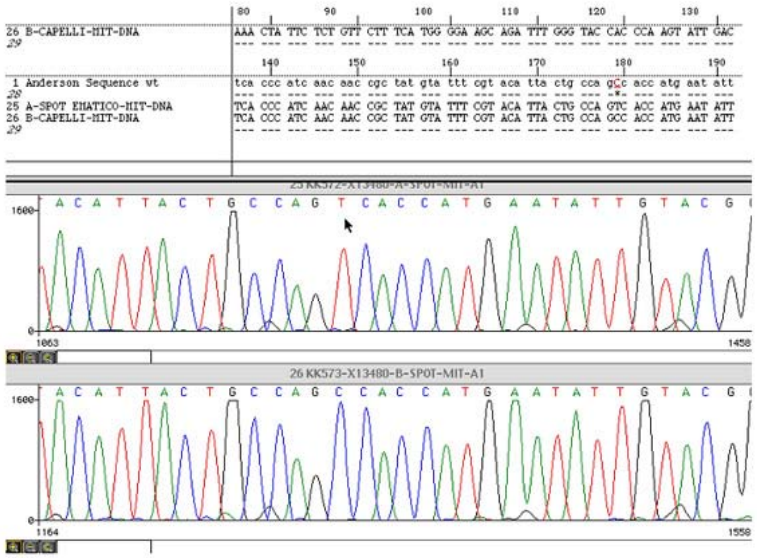


Fig. 14.4 The control of cryptobiosis: the effects of water presence, and humid or dry air externally, and of endogenous controls within the animal. OP, osmotic pressure.

https://www.youtube.com/watch?v=ejEUIVl9jXo

Transcriptomic approach: tardigrades as case study


26 B-CAPELLI-MIT-DNA
1 Anderson Sequence wt
25 A-SPOT EHATICO-MIT-DNA
26 B-CAPELLI-MIT-DNA
26 KK573-X13480-B-SPOT-MIT-A1
1063
1458
1164
1558

Desiccation treatment

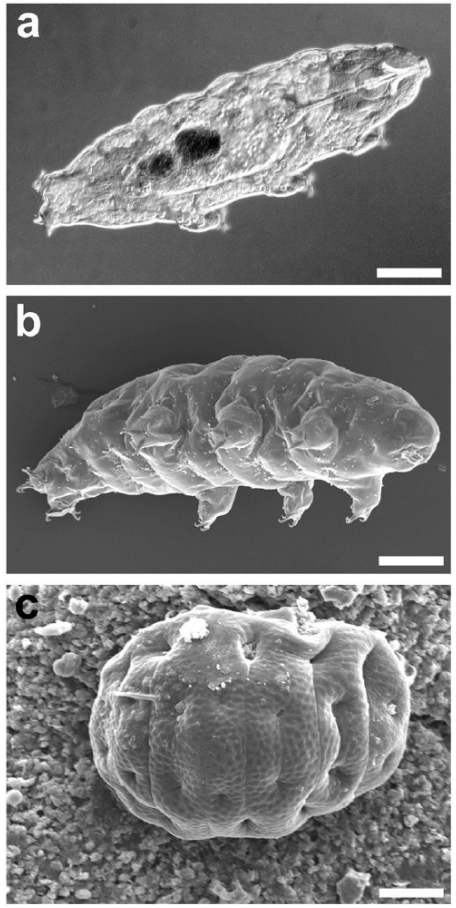
Each group of *P. sideralis* has placed on a Whatman filter paper (25 mm^2 or 1 cm^2) with mineral water (9 ml or 30 ml, respectively)

Exposition: initially to 80% relative humidity (RH) at 18°C for 4 hrs

then to 50% RH at 18°C for 4 hrs in a climatically controlled chamber

finally to 0%–3% RH at room temperature for 12 hr (Altiero et al., 2011).

At the end of the treatment animals exhibited the typical tun shape



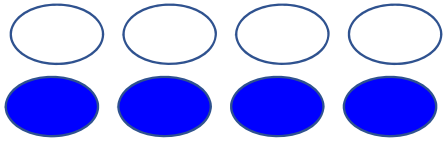
(a) Living specimens of *Macrobiotus sandrae* in active state (DIC). (b) Active *Richtersius coronifer* (SEM). (c) Dry *Ramazzottius oberhaeuseri* on lichen (SEM). Scale bars: a, b 1⁄4 50 mm, c 1⁄4 25 mm. (Guidetti et al., 2012)

Hydrated

Desiccated

RNA extraction

Total RNA



HiSeq 2000

Samples sequencing

tr2aacds pipeline

SignalP v4.0

Normalization and analysis

Stats on the transcriptome

Total assembled transcripts: 78.832 transcripts

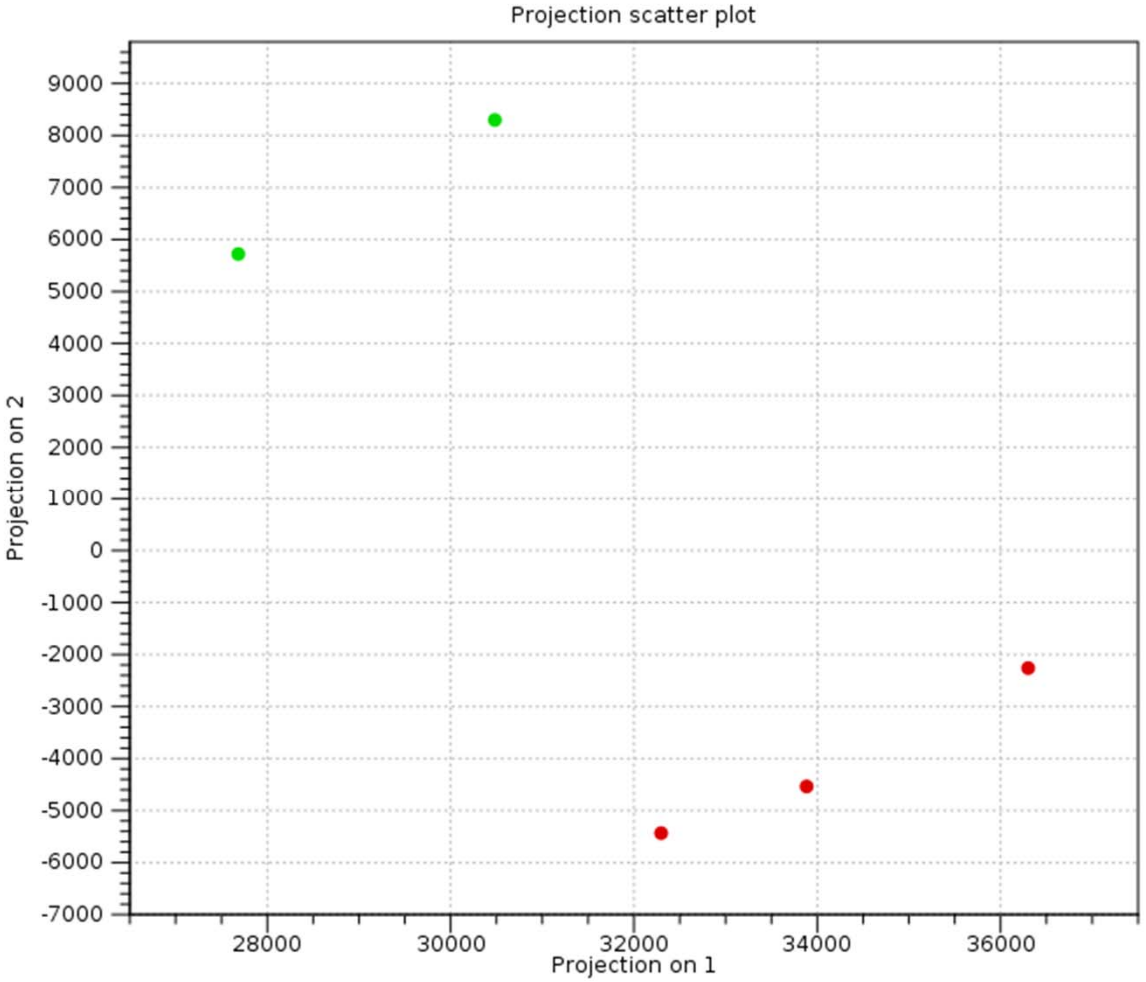
BUSCO v3

Assessing genome assembly and annotation completeness with
Benchmarking Universal Single-Copy Orthologs

C:85.5%[S:61.6%,D:23.9%],F:4.7%,M:9.8%,n:978

836 Complete BUSCOs (C)
602 Complete and single-copy BUSCOs (S)
234 Complete and duplicated BUSCOs (D)
46 Fragmented BUSCOs (F)
96 Missing BUSCOs (M)
978 Total BUSCO groups searched

Principal Component Analysis



- Desiccated
- Hydrated

Stats on the transcriptome

Total assembled transcripts: 78.832 transcripts

DEGs: FDR p-value <= 0,01
Fold change > 2

2.323 transcripts

UP

1710 transcripts (74%)

Among the highest 50 up-regulated DEGs with an enriched 10- to 200-fold during desiccation relative to hydrated conditions, **1/6th** resulted **unknown**.

DOWN

612 transcripts (26%)

Among the 50 highly suppressed transcripts, **half of them are unknown**.

Up-regulated transcripts

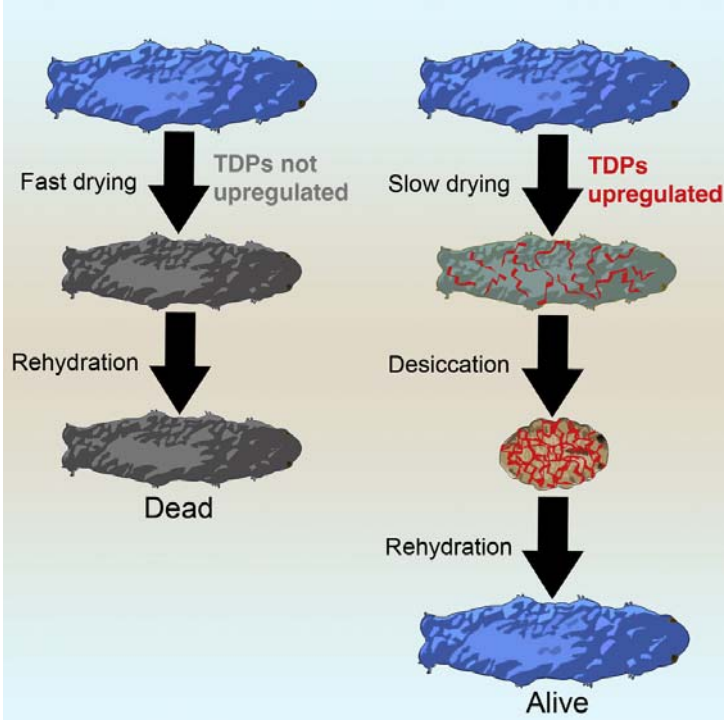
Transcript ID	Description	Log2 Fold Change	P-value
TRINITY_DN27205_c0_g1_i1	unknown	223,06	1,98E-07
TRINITY_DN26409_c0_g1_i1	unknown	195,98	3,75E-07
TRINITY_DN33882_c0_g5_i1	unknown	88,98	1,20E-05
TRINITY_DN32820_c0_g1_i7	unknown	62,02	5,62E-05
TRINITY_DN33032_c1_g2_i1	unknown	54,16	1,02E-04
TRINITY_DN28265_c0_g1_i17	Protein shuttle craft	34,93	1,09E-04
TRINITY_DN32168_c1_g1_i2	CAHS2	25,54	1,21E-09
TRINITY_DN32820_c0_g1_i1	Apolipophorins	25,51	1,01E-05
TRINITY_DN47949_c0_g1_i1	unknown	23,14	9,85E-06
TRINITY_DN18355_c0_g1_i2	Lysosome-associated membrane glycoprotein 1	22,19	1,03E-04
TRINITY_DN28724_c0_g1_i3	unknown	20,1	1,48E-09
TRINITY_DN38376_c0_g1_i7	CAHS2	20,13	1,39E-09

Summing up the enriched transcripts

- About 13% of them resulted to be secreted (54 to 2-fold).

- within the 50 highest induced and annotated transcripts we report :

5 Cytosolic-abundant heat soluble proteins (**CAHS**2s),
2 Apolipophorins,
2 Lysosome-associated membrane glycoproteins,
1 Annexin.



- among enriched DEGs:

37 CAHS transcripts
3 Soluble-abundant heat soluble proteins (SAHS) transcripts
2 HSP transcripts
2 Aquaporin transcripts
20 Annexin transcripts
6 trehalose phosphatase synthases involved in trehalose biosynthetic pathway

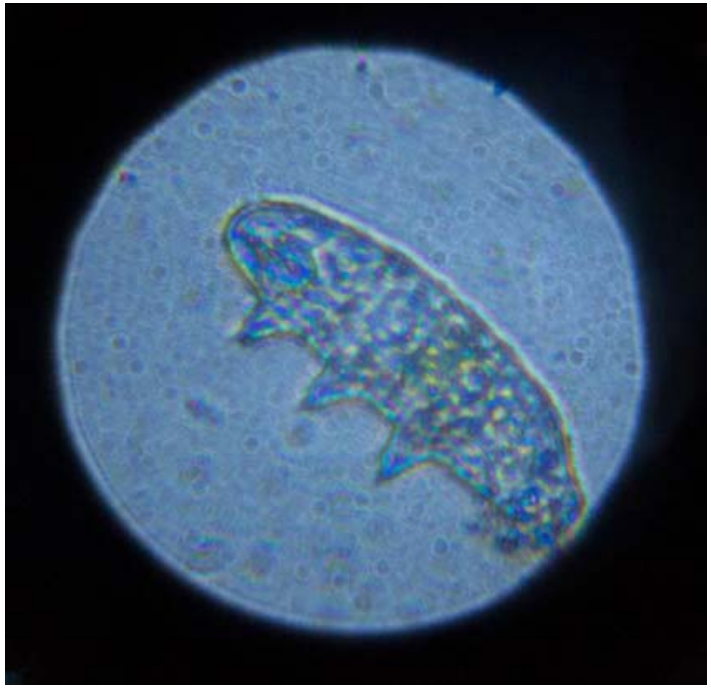
Down-regulated transcripts

Transcript ID	Description	Log2 Fold Change	P-value
TRINITY_DN38935_c0_g1_i13	Retinoid-inducible serine carboxypeptidase	-149,94	0
TRINITY_DN34279_c1_g1_i9	unknown	-145,74	0
TRINITY_DN29544_c0_g1_i1	unknown	-84,27	1,42E-06
TRINITY_DN30080_c0_g1_i1	Protein LSM14 homolog B	-61,13	1,86E-05
TRINITY_DN33429_c0_g1_i1	unknown	-60,52	9,86E-06
TRINITY_DN32814_c0_g1_i1	unknown	-54,03	1,83E-05
TRINITY_DN34403_c0_g1_i5	unknown	-52,85	2,22E-05
TRINITY_DN29450_c0_g2_i1	40S ribosomal protein S3a	-51,15	2,52E-05
TRINITY_DN32508_c1_g2_i3	unknown	-50,54	0
TRINITY_DN33883_c3_g2_i7	unknown	-45,4	4,69E-05
TRINITY_DN36566_c0_g2_i11	Apolipoprotein D	-44,05	4,10E-05
TRINITY_DN38824_c0_g3_i1	unknown	-41,73	6,48E-05

Summing up the suppressed transcripts

- About 20% of them are secreted (50 to 2-fold).
- Annotated categories are mainly related to **translation activity**, transketolase activity, and structural arrangement.
- Among suppressed DEGs:

  2 CAHS transcripts
  2 SAHS transcripts
  4 HSPs
  Apolipoproteins
  Actin-related proteins
  Cathepsins



Conclusions

- Confirmed activation of Intrinsically Disordered Proteins, (as CAHS) to survive desiccation (Boothby et al., 2017).

- Trehalose pathway seems also to be activated in *P. sideralis* as further desiccation-tolerance mechanism.

- Still several unknown transcripts deserve deeper investigation.

Lipidoma: i lipidi cambiano in risposta all'essiccazione. E ciò è importante perchè tutte le membrane biologiche sono fatte di lipidi. Sono utilizzati come membrane perché sono immersi in acqua. Togli l'acqua, e queste membrane si sgretolano. I lipidi inoltre fungono da segnali per catalizzare i geni.

Enhancer e Repressori