

Text S1

MATERIALS AND METHODS

The mudskippers were collected in the field (the place of origin is unknown), exported to Germany and sold in an aquaristics store in Salzgitter. Two male and 8 female *Periophthalmus variabilis* were purchased there on October 25, 2012 and kept in a paludarium (110 cm x 80 cm x 30 cm) equipped with a tidal system and a flat mud substrate of about 10cm thick. After the first larvae appeared, 1 male and 1 female (each about 90 mm long) were transferred to the experimentation tank (80 cm x 50 cm x 40 cm) on March 17, 2013. The second male and/or another female were later transferred there (Table S. 1). During the recording pauses the individuals were returned to the paludarium. Before recording resumed, 2 or 3 *P. variabilis* were again transferred to the experimentation tank. The experiment was completed on December 13, 2015. The tank was equipped with a tidal system that provided 16 hours of low tide (2:00 – 10:00 and 14:00 – 22:00) and 8 hours of high tide (10:00 – 14:00 and 22:00 – 2:00). An overflow marked the high-tide level and kept the water in motion, preventing the development of a microfilm on the water surface. Low tide prevailed for 8 hours when a second outlet was opened every 12 hours, using an electrically powered ball valve connected to an auto timer. Low tide in the experimentation tank thus automatically induced high tide in a basin below. Each tidal change took about 25 minutes. Before the water flowed into the basin below the tank, it was conducted into a basin with a constant water level, where a plankton net could trap hatched larvae (Fig. S1). The brackish water (salinity: 15 ppt, measured with a refractometer) was prepared with artificial sea salt and tap water and heated to 26⁰ C during daytime and to 24⁰C at night by an electrical heater inside the basin below the tank. The water was continuously pumped into the tank. The top of the tank was open so as to enable video recording. Therefore the air temperature corresponded to the room temperature (during winter month: about 22⁰C, during summer month up to 25⁰C). Water was only sponge-filtered, and evaporated water was replaced once a week. When the sponge filter was clogged, the water level rose slower than normal. Artificial light-dark cycles equaled 13 and 11 hours respectively. The tank was encased with black cardboard to minimize visual disturbance of the mudskippers. Because reproduction in a mud layer of 10cm had been successful, a mud slope 10 to 25 cm high was built inside the tank using natural mud from a North Sea beach. The substrate layer of about 25 cm in the experimentation tank seems to have been sufficient for burrowing. None of the 16 plaster casts of the burrows made during the course of the experiment extended the bottom of the aquarium. During low tide the water level in the tank was 10 cm. During high tide the water level was 30 cm, water covered the entire slope by about 5 cm over its peak. Parts of a large root and two bamboo sticks offered the mudskipper a place to sit above the water during high tide. Neap tides were experimented with during 5 breeding cycles: One week of normal tides with 2 low and 2 high tides per day alternated with 1 week without high tides.

The mudskippers were fed once a day in the evening during low tide with frozen bloodworms and Mysis, also occasionally with live Drosophila-flies and live brine shrimps. After a documentation phase with only short video clips by a Nikon D 90 digital camera, beginning on May 26, 2013 direct video recording was used to provide continuous documentation of breeding activity. During daylight, a Panasonic HC-V510 digital video cam recorder mounted over the tank was used. Three nocturnal high tides were also video-recorded. During more than 2.5 years, 42 breeding cycles of *P. variabilis* were observed and recorded. In this study a breeding cycle is the period characterized by the beginning of burrowing or entering the old burrow after a finished cycle, alternate diving of the male and female with subsequent male's

air adding behavior until larvae hatch (30 times) or the male's final leaving the burrow when no larvae hatched (12 times) (Table S1). Often, after the end of the breeding cycle, the male immediately started burrowing again. Video images of the entire mud slope were recorded. Some video footage focused on the 2 burrow entrances after the burrow location was selected and the male started burrowing. In the phase of the experiment when two males were together in the experimental tank, once both pairs were breeding at the same time and were video recorded together. Occasionally, the burrow was built directly adjacent to the glass wall of the tank. The black cardboard next to the burrow was removed and the video cam was placed near the side of the tank to video record the egg chamber and the vertical burrow shaft. In this manner 2 additional complete spawning phases were recorded (Table S1). On 1 occasion of these spawning phases the burrow opening was observed additionally from the outside with a Logitech HD Webcam C525 in order to distinguish the male from the female, which was stockier and a little bit paler than the male.

Table S1: Breeding cycles (- = 0, x = few, xx = < 100, xx = > 100, xxx = > 500, Ø = lost video material)

Breeding cycle	Date of hatching or male's permanent leaving of the burrow	Setting ♂,♀	Number of larvae
01	Ø	1,1	-
02	Ø	1,1	-
03	2013-05-05	1,1	-
04	2013-07-18	1,1	x
Recording pause			
05	2013-08-20	1,1	x
06	2013-09-14	1,1	xx
07	Ø	1,1	-
08	2013-09-29	1,1	x
09	2013-10-13	1,1	xx
Recording pause			
10	2013-11-27	2,1	xxx
11	2013-12-11	2,1	xxx
12	2014-01-23	2,1	xx
13	2014-02-07	2,2	xxx
14	2014-02-18	2,2	x
15	2014-02-20	2,2	-
Recording pause			
16	2014-03-17	1,2	-
17	2014-04-10	1,2	-
18	2014-05-06	1,2	xx
19	2014-05-19	1,2	-
20	2014-06-06	1,2	xxx
21	2014-06-17	1,2	-
22	2014-07-04	1,1	xxx
23	2014-07-15	1,1	xxx
24	2014-07-29	1,1	-
Recording pause			
25	2014-09-16	1,2	xxx
26	2014-09-26	1,2	-
27	2014-10-07	1,2	-
Recording pause			
28	2014-11-16	1,2	xxx
29	2014-12-06	1,2	xxx
30	2015-01-08	1,2	xxx
31	2015-01-26	1,1	xxx
32	2015-02-08	1,1	xxx
33	2015-02-22	1,1	xxx
34	2015-03-08	1,1	xxx
35	2015-03-19	1,1	xxx
36	2015-04-02	1,1	xxx
37	2015-04-15	1,1	x
38	2015-04-27	1,1	xxx
39	2015-05-21	1,1	xx
40	2015-06-06	1,1	xxx
41	2015-07-07	1,1	xxx
42	2015-09-14	1,1	xxx
Recording pause			
Spawning 01	Spawning date 2015-11-05	1,1	xx
Spawning 02	Spawning date 2015-11-27	1,1	xx

Table S2a: Video-recorded hatching events.

Video-recorded hatching events

	with tail-undulation phases	without tail- undulation phases
High tide after a series of alternating high and low tides	1	5
High tide after a series of neap tides	1	0

Table S2b: Video-recorded high tides with tail-undulation phases.

Video-recorded high tides with tail-undulation phases

Tides	with hatching event	without hatching event
High tide after a series of alternating high and low tides	1	19
High tide after a series of neap tides	1	8

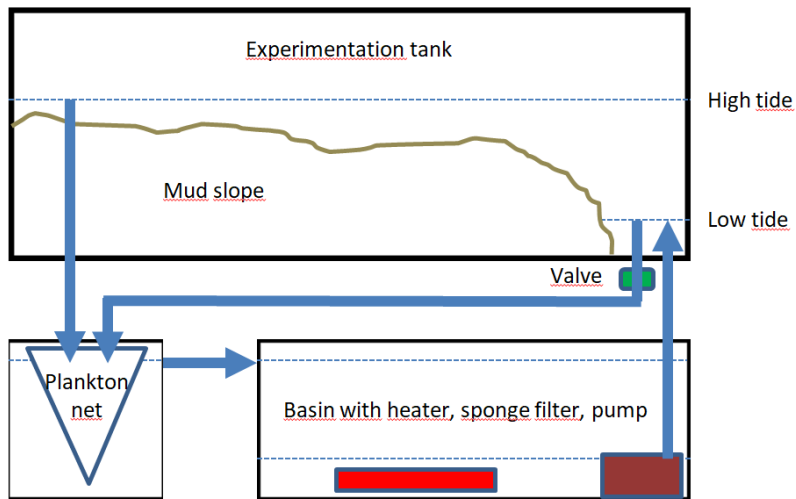


Fig. S1: Experimentation equipment



Fig. S2a: Male and female inside the U-shaped upper section of the burrow (2014-05-28, 18:53). Fig. S2b: The female left the burrow. Therefore, the water level should have decreased in comparison to Fig. S2a. However, the water level increased due to male's air addition into the egg chamber that displaced water there (2014-05-28, 21:28).

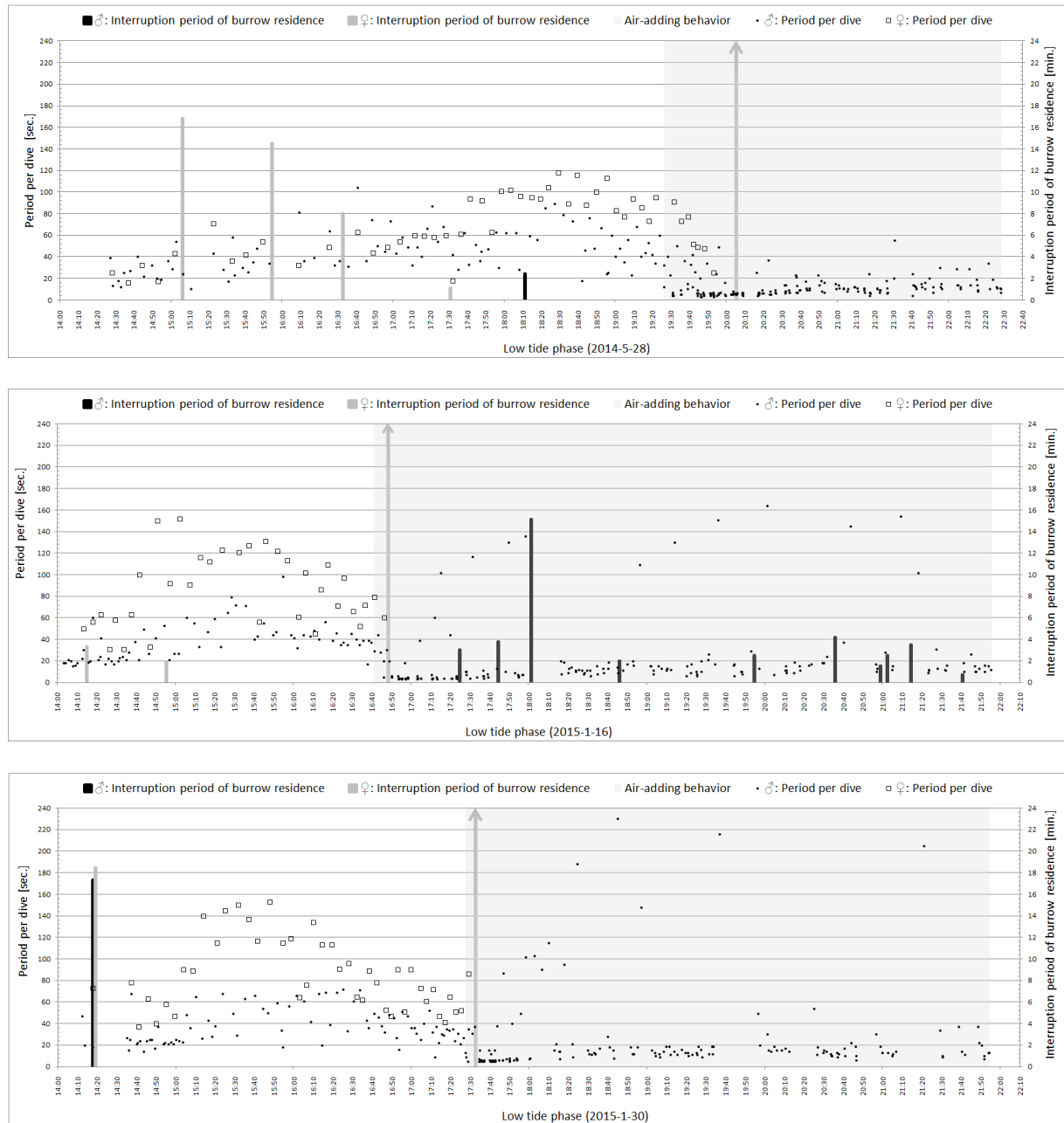


Fig. S3a-c: Duration of single dives and interruption periods of burrow residence of the female and the male during three reproduction events (↑ permanent burrow leaving of the female).

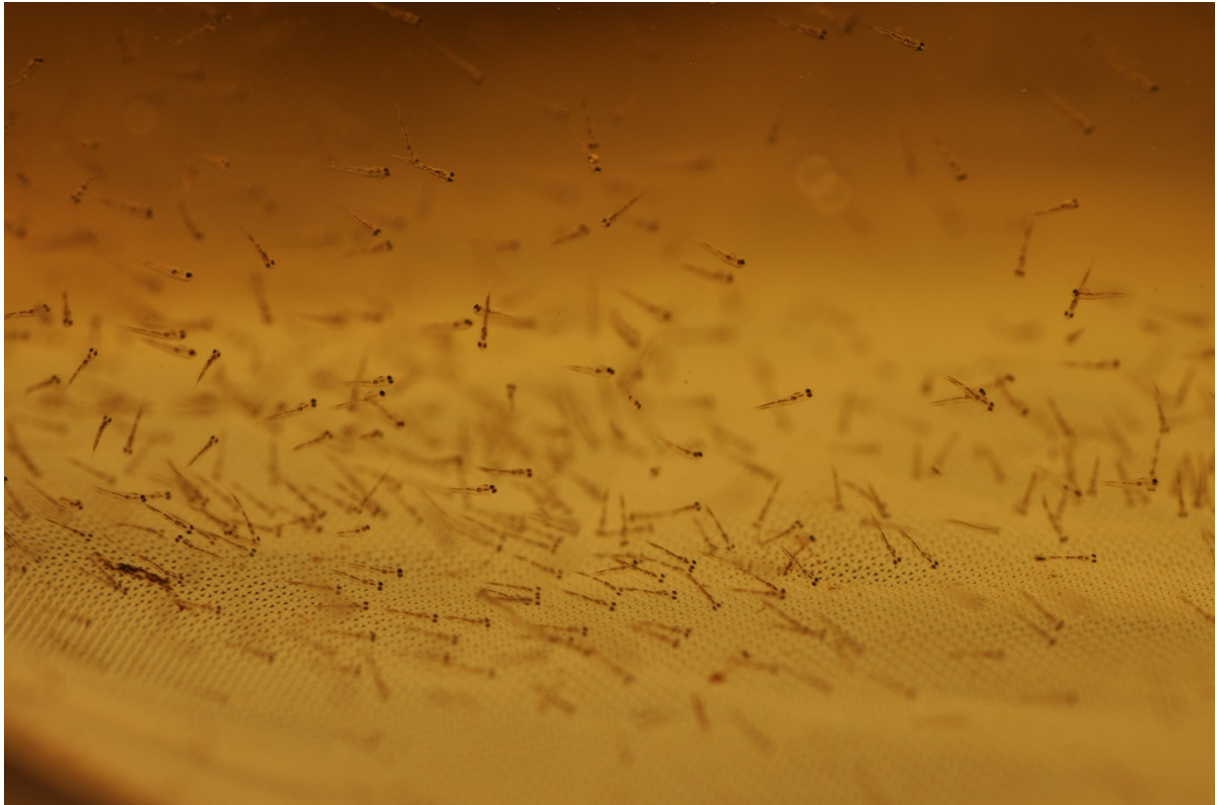


Fig. S4: Mass hatching. All larvae were trapped in the plankton net.

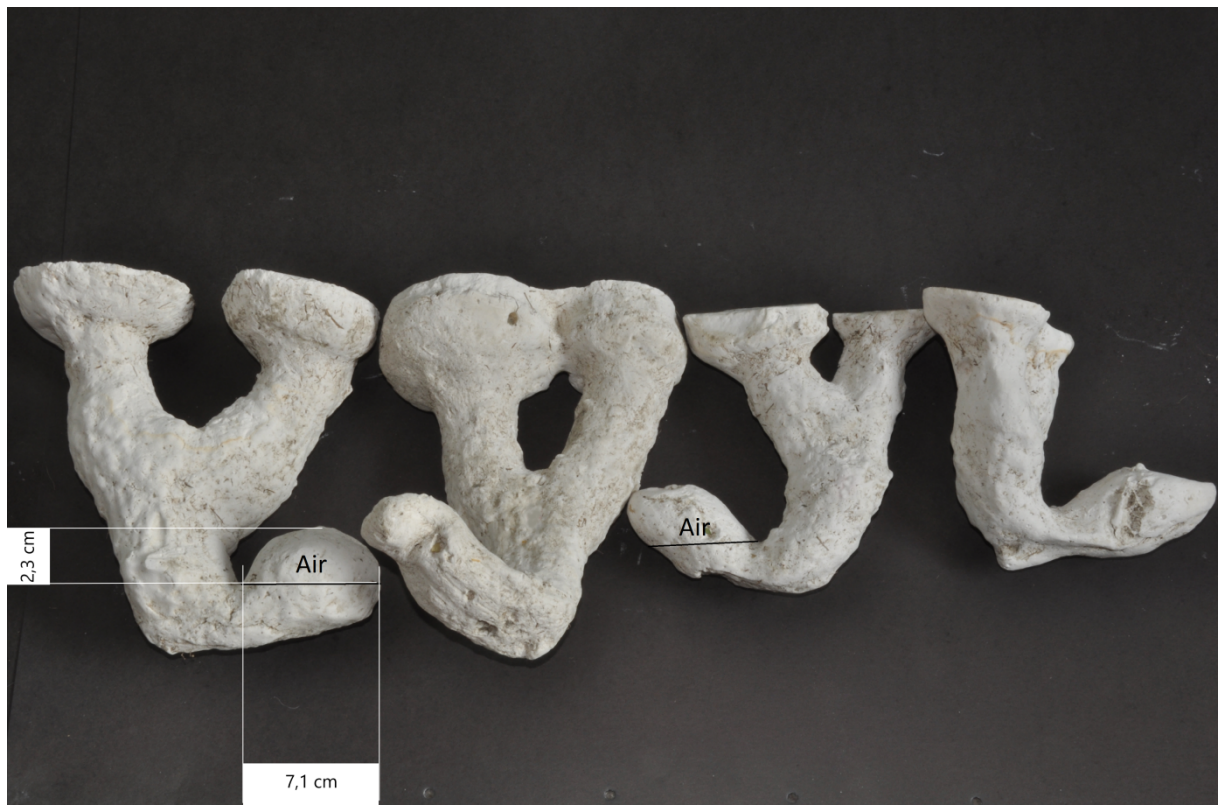


Fig. S5: Plaster casts of burrows of *P. variabilis*, 2 of which showing the possibility of air storage.

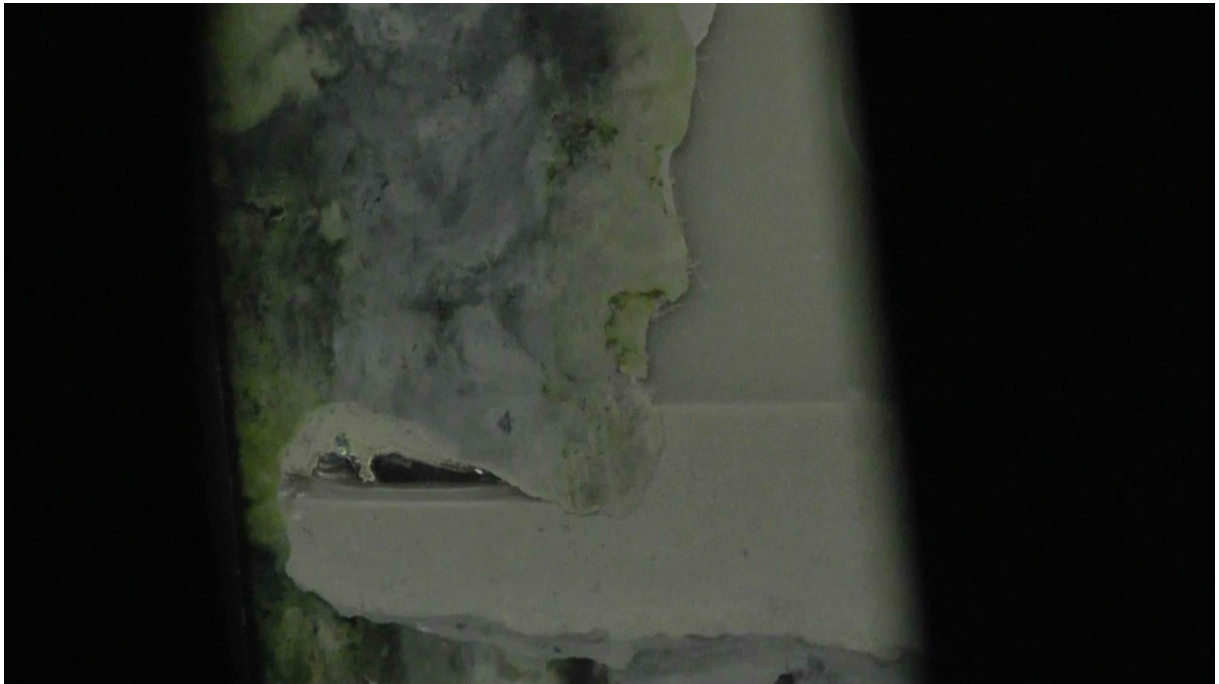


Fig. S6: Air-addition has progressed far. Eggs can be clearly recognized in front of the dark, air-filled background. The male stays inside the egg chamber.

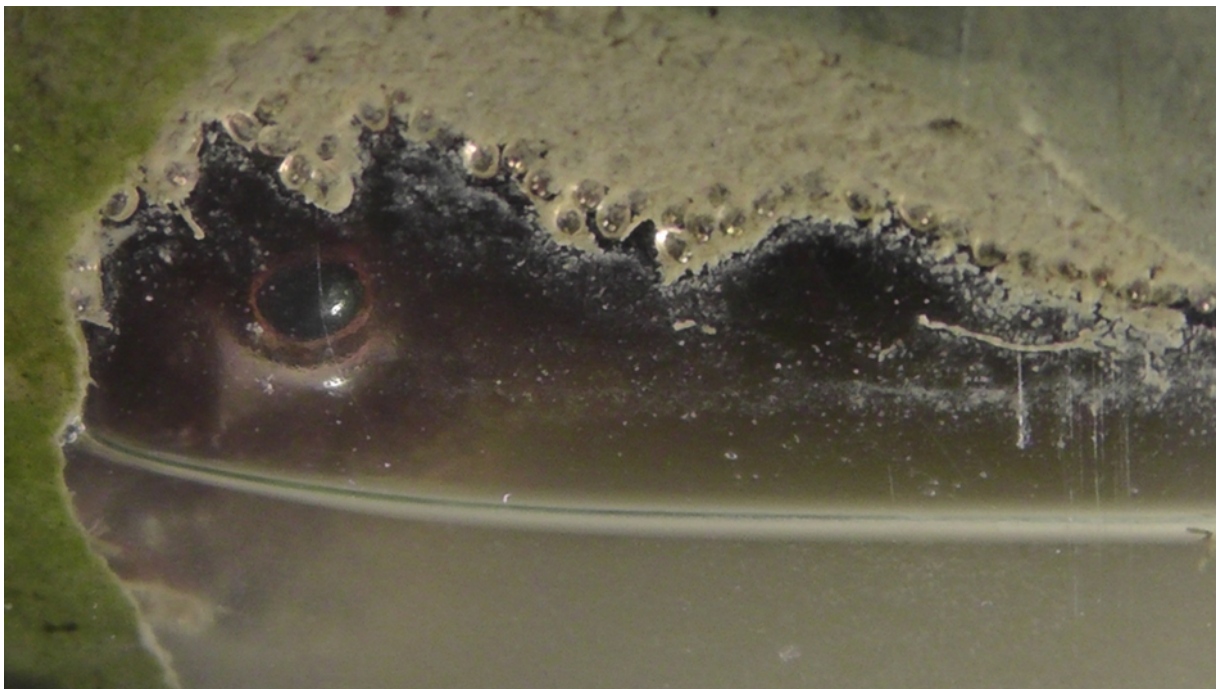


Fig. S7: Larvae development (i.e. larvae eyes) can be seen. The male is inside the egg chamber.

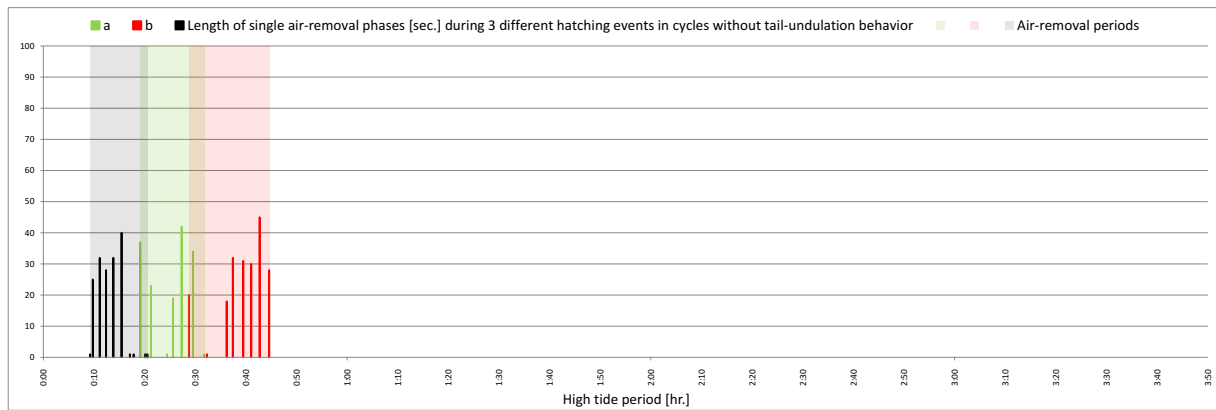


Fig. S8: Length of single air-removal phases [sec.] and duration of air-removal behavior within 3 evening hatching events in breeding cycles without tail-undulation behavior.

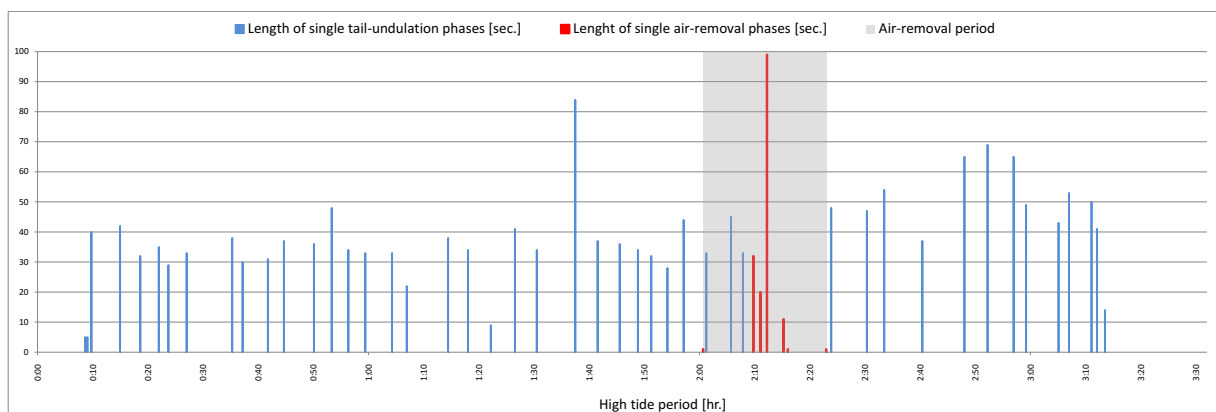


Fig. S9: Length of single air-removal phases [sec.] and single tail-undulation phases [sec.] during 1 nocturnal hatching event.

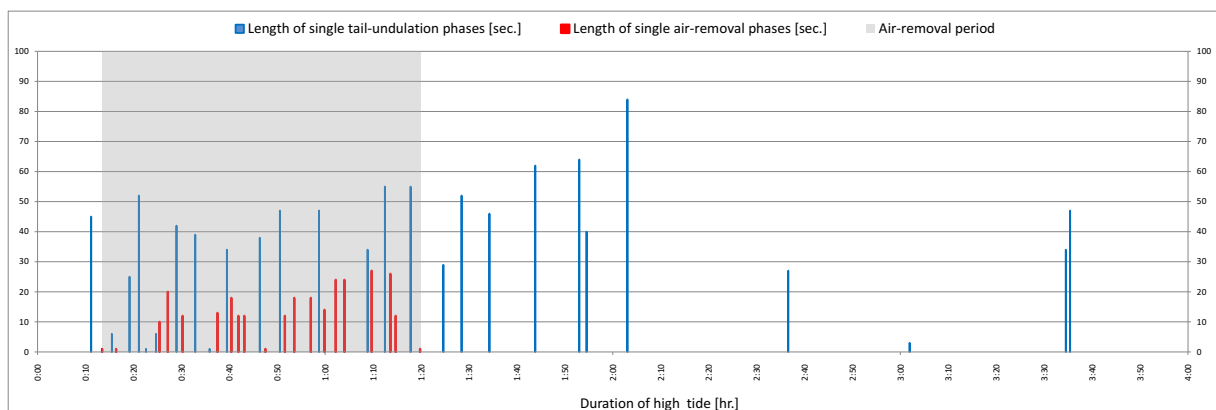


Fig. S10: Length of single air-removal phases [sec.] and single tail-undulation phases [sec.] during 1 morning hatching event on the first high tide after a phase of neap tides.

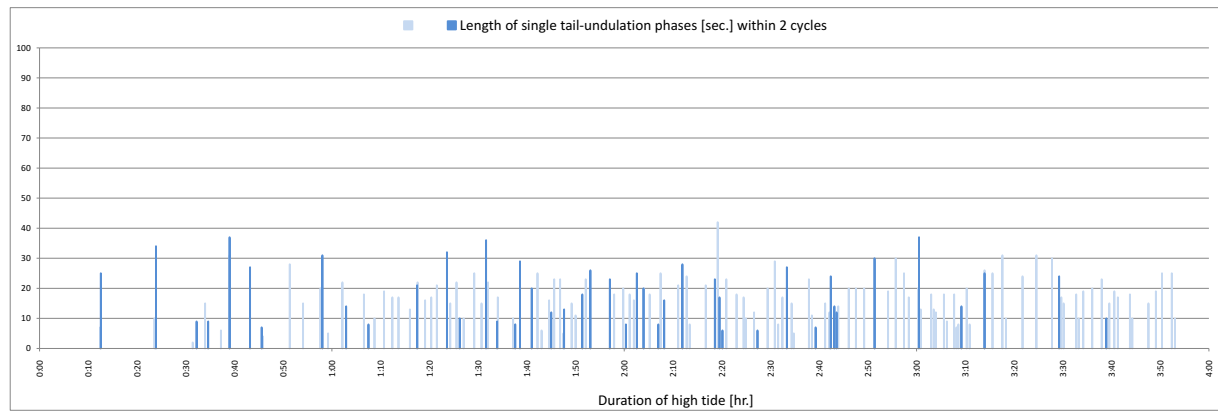


Fig. S11: Duration of single tail-undulation phases within 2 different breeding cycles, each on the first high tide after a phase of neap tides, both without hatching event.

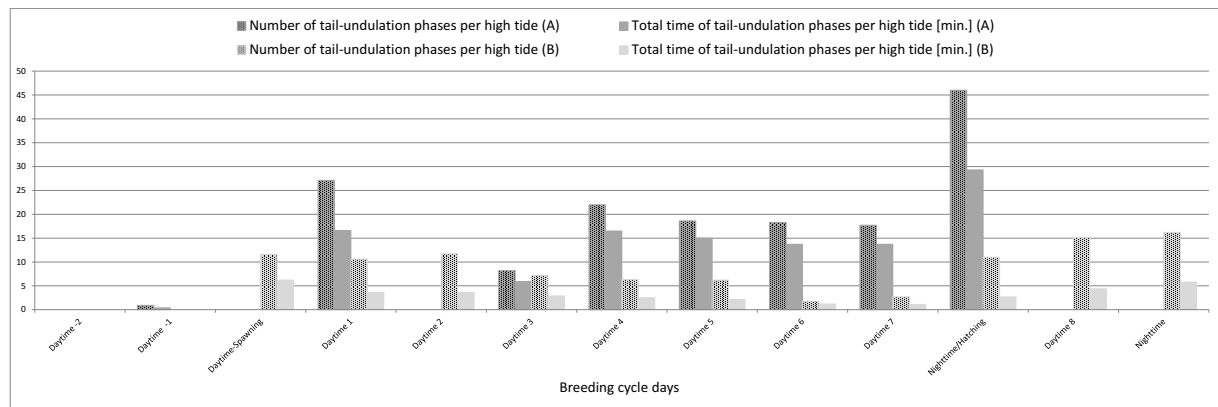


Fig. S12: Number and total time of single tail-undulation phases per high tide of 2 breeding cycles (A: with nocturnal hatching, B without hatching event).