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Increased settlement rates of field-caught barnacle larvae in settlement assays adding metamorphosed juveniles

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FOUL PROTECT

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Introduction

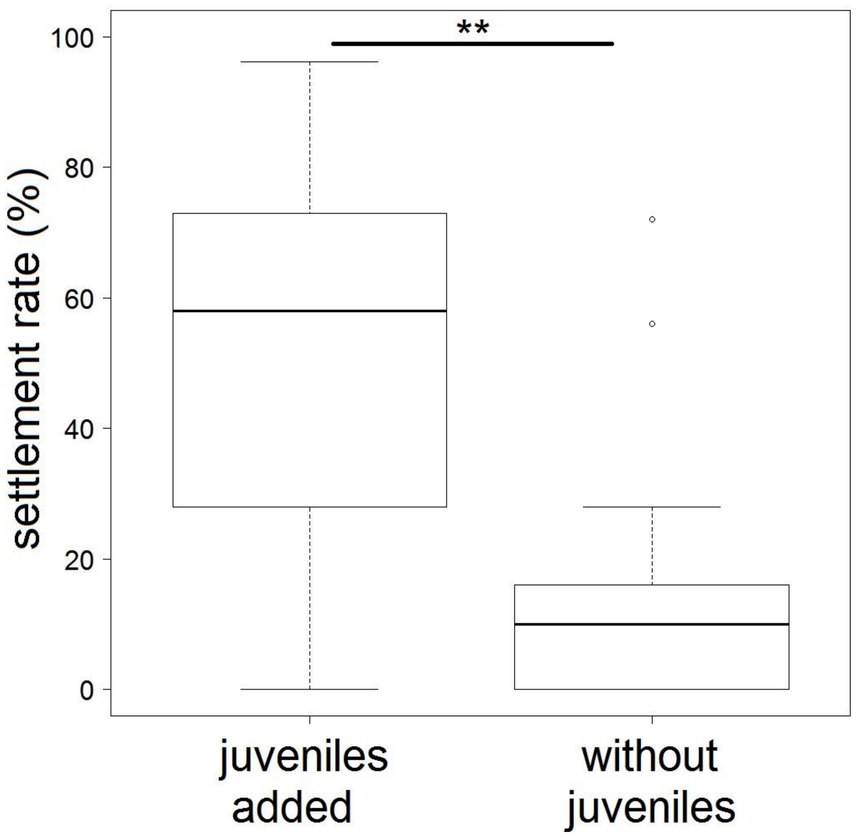
Barnacles are known to cause severe problems in the shipping industry and industrial aquatic processes due to their settlement on ship hulls and other underwater constructions (Fig. 1). Settlement on ship hulls can result in an increase of fuel consumption and operational costs as well as shortened dry dock intervals may lead to increasing operational costs.
Barnacles are often used as a model organism in antifouling research to examine the efficacy of new antifouling substances by testing the prevention of barnacle fouling in laboratory bioassays. However, in many assays settlement rates of barnacle larvae were quite low [1, 2]. The aim of this study was to increase the settlement rate and the reliability of a settlement assay with cypris larvae of the species *Austrominius modestus* (Darwin, 1854) grown in the field.

Fig. 1 Settled barnacles of the species *Austrominius modestus* in various size classes (left), juvenile barnacle filtering water (middle) and cyprids with compound eyes and oil cells (right).

Model organism

The invasive and competitive species *A. modestus,* which spread widely in European waters since their invasion in the 1940s, shows high resistance to variations in temperature and salinity. After six stages of nauplius larvae the non-feeding cypris stage represents the last phase before permanent attachment to a suitable surface (Fig. 3) and requires a thorough decision concerning a suitable habitat for the rest of the barnacles' life. Their ability to survive without food up to several weeks and to explore the potential substrate by their antennules make them a perfect model organism.
Larvae were collected in the harbour of the island Norderney, North Sea, with a plankton net with a mesh size of 55 µm. They were stored in darkness at 6°C until the beginning of the tests. 25 larvae were put in each petri dish in addition to five metamorphosed juveniles. Juvenile barnacles after settlement derived from petri dishes or were collected from panels exposed in the field for a few days. In both cases, the membranous basal plate of the juveniles was still flexible enough to remove the barnacles without killing them. A total of 34 petri dishes containing cypris larvae were tested. After one week test duration, settled larvae per petri dish were counted and compared with results from control petri dishes without added juveniles.

Results



- all larvae were alive and able to swim (cyprid) or to filter water (metamorphosed juvenile) after the end of the test
- mean settlement rates:
  - without juveniles: 16,57 ± 22,01%
  - with added juveniles: 52.50 ± 30.01%
- after one week settlement rates up to 96% were reached in petri dishes with added juveniles (Fig. 2)
- settlement rates between petri dishes with and without added juveniles differed significantly ($p = 0.0012$)

Fig. 2 Box-and-Whisker-Plots of settlement rates of barnacle cyprid larvae in tests with added juveniles and without juveniles (n=34). Significance level $p < 0.05$, Mann–Whitney *U* test.

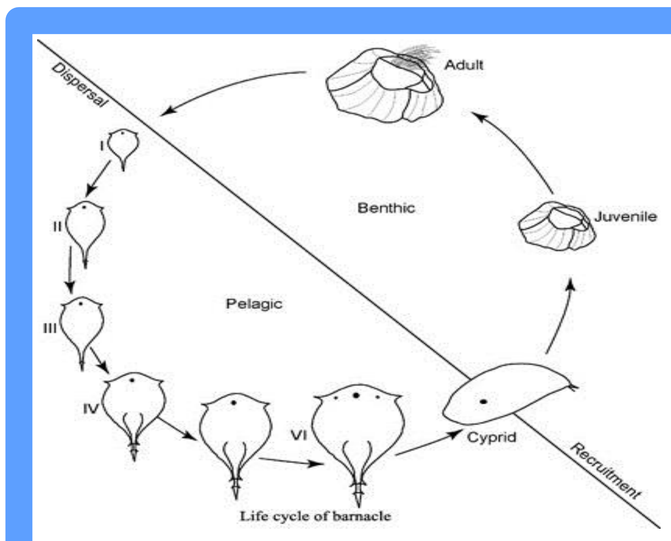


Fig 3 Stages in a life cycle of a typical balanomorph barnacle (Family Balanidae) with six stages of nauplius larvae and the last larval stage of a cyprid.
Figure adapted from D.V. Desai, A.C. Anil, *J. Mar. Biol. Assoc*. UK. **2005,** 85, pp. 909-920.

Discussion

Increased settlement rates in petri dishes containing metamorphosed juveniles can be explained by the release of chemical substances by adult barnacles [3]. This natural process is assumed to ensure the settlement of cypris larvae close to existing colonies in favour of enhanced reproduction.
The settlement assay as described here is quite suitable to test the efficacy of new antifouling paints in the laboratory. However, the test cannot be performed all year around since rearing of larvae in barnacle cultures is not intended in our laboratory. Furthermore, catching and sorting of the larvae grown in the field was very laborious and time-consuming.
Observations on an offshore station on the island Norderney showed that the settlement of barnacle larvae can occur within the first 24 hours after exposure. Therefore, a cypris larvae rapid test was developed as an alternative test method in the field (Fig. 4): Three replicates of micro slides were exposed to field conditions restricted to barnacle larvae. After one week settled barnacles were counted and compared to control coatings. The test method offers reliable results in a short period of time and provides an inexpensive test method to get first results about the efficacy of new antifouling products. Both methods are offered at the marine station of the Laboratory for Freshwater and Marine Research, LimnoMar, and can be conducted during the summer season.

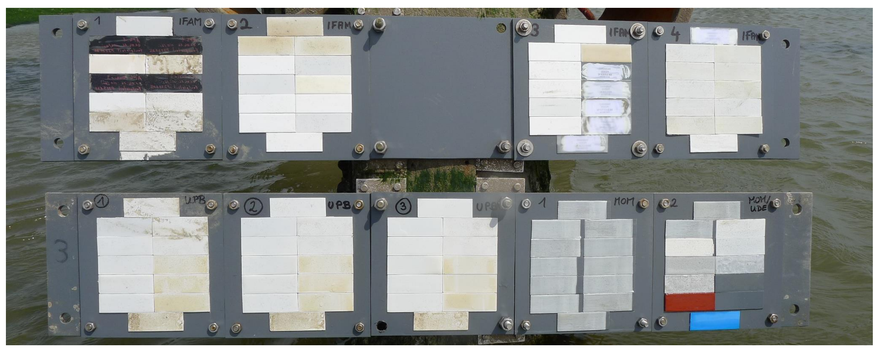


Fig. 4 Cypris larvae rapid test at the seaside of Norderney with micro slides exposed to field conditions.

References

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