

# Being young in a changing world: how temperature and salinity changes interactively modify the performance of larval stages of the barnacle *Amphibalanus improvisus*

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**Abstract** The fate of key species, such as the barnacle *Amphibalanus improvisus*, in the course of global change is of particular interest since any change in their abundance and/or performance may entail community-wide effects. In the fluctuating Western Baltic, species typically experience a broad range of environmental conditions, which may pre-select them to better cope with climate change. In this study, we examined the sensitivity of two crucial ontogenetic phases (naupliar, cypris) of the barnacle toward a range of temperature (12, 20, and 28°C) and salinity (5, 15, and 30 psu) combinations. Under all salinity treatments, nauplii developed faster at intermediate and high temperatures. Cyprid metamorphosis success, in contrast, was interactively impacted by temperature and salinity. Survival of nauplii decreased with increasing salinity under all temperature treatments. Highest settlement rates occurred at the intermediate temperature and salinity combination, i.e., 20°C and 15 psu. Settlement success of “naive” cyprids, i.e., when nauplii were raised in the absence of stress (20°C/15 psu), was less impacted by stressful temperature/salinity combinations than that of cyprids with a stress history. Here, settlement success was highest at 30 psu particularly at low and high temperatures. Surprisingly, larval survival was not highest under the conditions typical for the Kiel Fjord at the season of peak settlement (20°C/15 psu). The proportion of nauplii that ultimately transformed to

attached juveniles was, however, highest under these “home” conditions. Overall, only particularly stressful combinations of temperature and salinity substantially reduced larval performance and development. Given more time for adaptation, the relatively smooth climate shifts predicted will probably not dramatically affect this species.

## Introduction

Intertidal barnacles as conspicuous, widely distributed, and ecologically important species have served to study the effects of environmental factors, e.g., temperature, salinity, food type and concentration, light (e.g., Crisp and Ritz 1973; Harms 1984; Holm 1990; Konya and Miki 1994; Anil et al. 1995, 2001; Anil and Kurian 1996; Qiu and Qian 1997, 1999; Hentschel and Emlet 2000; Thiyagarajan et al. 2002a; Nasrolahi et al. 2007), and more recently global warming and ocean acidification (Findlay et al. 2008; Hung et al. 2008; Findlay et al. 2009; McDonald et al. 2009; Wong et al., in press) on larval development and settlement success. Much of this progress on what is frequently considered the most sensitive life history phase is facilitated because barnacle larvae can be easily reared under controlled conditions (Thiyagarajan et al. 2000). Thus, numerous studies of stress impacts have been conducted using the widespread barnacle *A. amphitrite* (e.g., Anil et al. 2001; Desai and Anil 2002, 2004; Desai et al. 2006; McDonald et al. 2009) and other locally common barnacles species, e.g., *Balanus trigonus* (Thiyagarajan et al. 2003a), *Balanus glandula* (Berger 2009), *A. improvisus* (Nasrolahi et al. 2006), and *Semibalanus balanoides* (Pineda et al. 2002; Findlay et al. 2010).

It is well accepted by now that in the course of global change, several potential stressors will appear and intensify simultaneously and may exhibit antagonistic, additive, or

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synergistic effects, which make predictions based on the assessment of single stressors unreliable (e.g., Wahl et al. 2011). The interactive effect of putative stresses such as high temperature and low salinity on larval performance and survival of barnacles has, however, been subject of only a few studies (Harms 1986; Anil et al. 1995; Qiu and Qian 1999; Thiyagarajan et al. 2003b). Responses toward multiple stressors of *A. improvisus*, the dominant barnacle species within the Western Baltic Sea, have not been investigated so far, neither in adult nor in larval stages.

The energy content of cyprids depends on the previous planktotrophic naupliar development (Pechenik 1987; Anil et al. 2001; Thiyagarajan et al. 2002b, 2003b) and thus, not only stressors may interact, but also their impact on successive developmental stages. This development is determined by various environmental factors such as temperature, salinity, and availability of food (Anil et al. 1995, 2001; Anil and Kurian 1996; Desai et al. 2006). Consequently, the stress acting during early larval development might impact the performance and stress sensitivity of later stages, another aspect relatively neglected so far (but see Qiu and Qian 1999).

When stress impacts a foundational species, it may percolate through the community easily (e.g., Wahl et al. 2011). *Amphibalanus improvisus* is such a common, locally dominant, and ecologically important fouling organism in the Western Baltic (Dürr and Wahl 2004). While its recruitment peaks in the summer (June–September), lower numbers of recruits can be encountered throughout the year (personal observation). Due to small-scale spatial and seasonal environmental variability, larvae within the Western Baltic may experience a broad range of salinities and temperatures during different months of their occurrence, at different locations and even at different depths. In Kiel Fjord, the site of the investigated barnacle population, salinity fluctuates between 5 and 20 psu at the surface and between 16 and 23 psu at the bottom (Madhupratap et al. 1996, personal observation). Sea surface temperature (SST) can easily reach from 0°C in winter to up to 23°C during late summer (Baumann et al. 2007). In coastal lagoons and in shallow waters, the temperature can even be several degrees higher as shown in 1 m depth at the Schlei Fjord (Kiel Bight, Western Baltic) with a maximum temperature of 27°C (K. Maczassek unpublished data). During *A. improvisus* peak recruitment from July to August (Thomsen et al. 2010), SST in Kiel Fjord typically is around 20°C and surface salinity is about 15 psu (own unpublished logger data). These conditions, if not optimal, can at least be considered benign.

Although the rearing conditions may influence the performance of subsequent stages such as cyprids or post-larvae (Pechenik et al. 1993; Qiu and Qian 1999; Pechenik 2006; McDonald et al. 2009), earlier studies have mainly

examined the settlement success of cyprids without a stress history (e.g., Dineen and Hines 1992; Thiyagarajan et al. 2003a; Dahlstrom et al. 2004; McDonald et al. 2009). In this study, we focus on three understudied aspects of stress ecology: the interaction of stressors, their impact on early life stages, and the influence of stress history on subsequent life stages.

## Materials and methods

### Rearing of barnacle larvae

Pebbles and stones bearing adult *A. improvisus* were collected from Kiel Fjord (Western Baltic) in summer 2009 and transferred to the laboratory. After rinsing with seawater, they were placed in seawater-filled trays at a constant temperature of 20°C under a 12:12 h day/night light cycle and provided with a flow through of filtered fjord water. Barnacles were fed daily on a diet of freshly hatched brine shrimp (*Artemia salina*). Adults released nauplii after few days, which were collected in sieves (90 µm) from the tray overflow. These nauplii were at stage I and developed into stage II within few hours. Mixed batches of stage II larvae from multiple parents were used for the two experiments. The life cycle of barnacles includes six planktotrophic naupliar larval stages followed by a non-feeding, planktonic cypris (Honglei et al. 2010).

### Experiment I

The influences of the combination of temperature and salinity on various aspects of larval performance were assessed during the 4-week span until larvae metamorphosed and settled. Response variables were (1) total larval duration (days from hatching until 50% of the surviving nauplii had developed into cyprids), (2) survival (percent surviving nauplii or cyprids relative to the respective initial numbers), (3) percentage of cyprids (percent of the initially introduced 20 nauplii per well, which had turned into cyprids by the given time of the experiment), and (4) settlement (percent of settled juvenile barnacles relative to initial number of nauplii or relative to the number of surviving cyprids).

We used three different salinities (5, 15, and 30 psu) and three different temperatures (12, 20, and 28°C) in a fully mixed experimental design. The different salinities used in the experiment were obtained by diluting filtered (0.2 µm) artificial seawater (30 psu) with double distilled water. Although diluting seawater with distilled water may create unnaturally low alkalinities at low salinity values, this is a common method used in salinity assays on barnacle larvae (e.g., Qiu and Qian 1999; Thiyagarajan et al. 2003a; Tindle et al. 2004). Personal measurement in our study, however,

showed that alkalinity decreased with diluting seawater in a natural range as measured by Beldowski et al. (2010) for the Baltic Sea at different salinity values. Each treatment combination consisted of eight replicates (one thermo bath for each treatment) with 20 larvae per replicate (one individual well per replicate, see below). Cultures were kept in thermo bathes (Thermostat HAAKE DC10, Karlsruhe, Germany) adjusted to the target temperatures ( $\pm 0.1^\circ\text{C}$ ).

Larvae were transferred into six-well plates (CELL STAR # 657160) at the respective salinities (defined per well) and temperatures (defined per plate in a given thermo bath). Marine diatoms *Chaetoceros calcitrans* and *Skeletonema costatum* were grown in f/2 medium ( $15^\circ\text{C}$  and 15 psu) and used as larval food. For each treatment, 20 larvae were incubated in 10 ml of filtered ( $0.2\ \mu\text{m}$ ) seawater (FSW) and were fed daily with 50:50 mixture of both diatom species at  $2 \times 10^5$  cells  $\text{ml}^{-1}$ . Larval cultures were kept under continuous light ( $16\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ ) to minimize mortality and precipitation of algal cells (Thiyagarajan et al. 2003a, 2003b). Every 24 h, larvae were observed using a binocular microscope (WILD M3C Heerbrugg, Switzerland) and the number of survivors, percentage of cyprids, and settlement were recorded for each replicate. Since the counting was fast, the water temperature in each treatment did not change noticeably during observation under the binocular. Hereafter, all cyprids reared in this experiment (Exp. I) are called “pre-stressed” regardless of the fact that some of them were reared in the ambient, i.e., no-stress conditions ( $20^\circ\text{C}/15$  psu). The water and food in the wells were replaced every second day by fresh seawater with the respective temperature and salinity. To keep the temperature constant for each treatment while changing the water in the wells, preconditioned water was used from 2 l water bottles that were kept in thermo bathes with the respective temperature.

## Experiment II

Nauplius II larvae (new batch) were obtained as in Experiment I and kept at a density of 1 larva per 2 ml filtered ( $0.2\ \mu\text{m}$ ) seawater in 20-l buckets at 15 psu. Each bucket was provided with a mixture of *C. calcitrans* and *S. costatum* as described for Experiment I. The buckets were kept in a water bath equipped with circulation and an immersion heater at  $20^\circ\text{C}$ . The cultures in the buckets were gently aerated, while seawater was replaced and microalgae were provided every third day. When cyprids appeared in the cultures after 7–8 days, the cultures were sieved ( $200\ \mu\text{m}$ ) and cyprids were removed and subsequently used for settlement bioassays. As in Kiel Fjord, settlement peak usually occurs in July and August (mean temperatures of about  $20^\circ\text{C}$  and the mean salinities of 15 psu); These cyprids were reared under conditions as prevail in Kiel Fjord during peak

settlement time (habitat condition) (Thomsen et al. 2010) and thus, presumably, without temperature and salinity stress.

The levels of temperature and salinity tested were the same as in Experiment I. 10 cyprids and 5 ml of FSW were transferred into sterile six-well plates with different salinities and incubated at the different temperatures under continuous light. The transition from starting to target conditions was done gradually (1 psu/h,  $1^\circ\text{C}/2$  h) to allow for acclimatization. Settlement and survival of cyprids were monitored daily over 25 days. Every second day, seawater in experimental containers was replaced. Four replicates were used for each treatment.

## Statistical analysis

The majority of data sets were not normal due to the frequent observation of zeros. This is an inherent property of the response variables we collected: no cyprid metamorphosis/settlement during early phases of the experiment resulted in strongly skewed frequency distributions, which could not be transformed to normality. Therefore, we refrained from applying parametric statistical procedures and employed permutational multivariate analysis of variance (PERMANOVA) instead. Analyses were undertaken using the PERMANOVA+ add-on for PRIMER 6.0 (Anderson et al. 2008) for elucidating the effects of temperature and salinity on the different response variables we measured. This test procedure provides values of  $p$  from permutations (4,999 in all our tests), which we based on binomial deviance dissimilarities. This measure is appropriate for this kind of empirical distributions (Anderson and Millar 2004), and we derived it from the non-transformed data. Our PERMANOVA design had two fixed factors (“Temperature,” 3 levels; “Salinity,” 3 levels) and the interaction term between these two. Since we sampled our experimental units repeatedly, we viewed the different sampling days as dependent variables and thus employed the multivariate approach. PERMANOVA does not assume any underlying data distribution, but is sensitive to differences in dispersion across samples. We tested for this using the PERMDISP routine in PERMANOVA+ and found it given for the majority of our tests. However, since PERMANOVA is robust toward violations of the homogeneity of dispersion, we continued with the analysis even in those cases when dispersion among groups was not homogenous (Anderson et al. 2008). The effects of temperature and salinity on total larval duration and final settlement were evaluated using parametric two-way ANOVAs (Table 1). For this, we focused on the last day of the experiment and ignored all previous samplings. Furthermore, we ran a  $t$  test to test for a difference among the 2 larval batches used in the two subsequent experiments regarding mean settlement

**Table 1** Exp. I (with pretreated larvae): effects of temperature and salinity on nauplii to cyprid metamorphosis success (a), survival (b), and cyprid settlement from nauplii (c) in *Amphibalanus improvisus*

Source	df	SS	MS	Pseudo- <i>F</i>	<i>p</i> (perm)	perms
<i>(a) Nauplii to cyprid metamorphosis success</i>						
Te	2	1,765.1	882.54	58.356	0.0002	4,988
Sa	2	344.12	172.06	11.377	0.0002	4,989
TexSa	4	144.93	36.232	2.3958	0.0254	4,979
Res	63	952.77	15.123			
Total	71	3,206.9				
<i>(b) Survival</i>						
Te	2	45.521	22.76	1.5214	0.2088	4,986
Sa	2	390.64	195.32	13.056	0.0002	4,989
TexSa	4	74.54	18.635	1.2456	0.2872	4,984
Res	63	942.52	14.961			
Total	71	1453.2				
<i>(c) Settlement from 20 nauplii</i>						
Te	2	672.52	336.26	18.091	0.0002	4,988
Sa	2	183.38	91.688	4.933	0.0084	4,990
TexSa	4	144.95	36.237	1.9496	0.1006	4,983
Res	63	1,171	18.587			
Total	71	2,171.8				

Results from multifactorial PERMANOVA

PERMANOVAs were based on the binomial dissimilarity measure. *p* values were obtained using 4,999 permutations of residuals under a reduced model

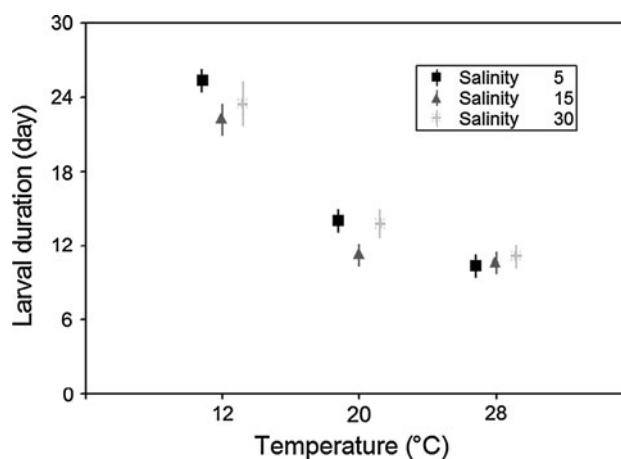
rates under no-stress conditions (15 psu, 20°C). We verified normality of data with Shapiro–Wilk’s *W* test and explored homogeneity of variances with Levene’s test. In case of deviations from the assumptions, we applied appropriate transformations prior to the analyses.

## Results

### Experiment I

The time required for larvae to develop from nauplius II to cypris stage (Exp. I) was almost twice as long at 12°C than at 20°C and 28°C, but did not differ substantially between the two latter temperatures (Fig. 1). The effect of temperature on total larval duration was significant (ANOVA,  $F = 97.02$ ,  $df = 2$ ,  $p < 0.001$ ), while the effect of salinity (ANOVA,  $F = 2.93$ ,  $df = 2$ ,  $p = 0.06$ ) and its interaction with temperature (ANOVA,  $F = 1.11$ ,  $df = 4$ ,  $p = 0.35$ ) was not statistically significant.

The rate of successful metamorphosis of nauplii to cyprids (Exp. I) was interactively impacted by temperature and salinity (Table 1a). At 5 psu, the percentage of successful metamorphosis was highest at all temperature levels most conspicuously so at 28°C (Fig. 2). Metamorphosis was



**Fig. 1** *Amphibalanus improvisus*. Effect of temperature and salinity on total larval duration. Each bar represents the mean ( $\pm$ SE) of eight replicates. Each replicate consisted of 20 nauplius larvae

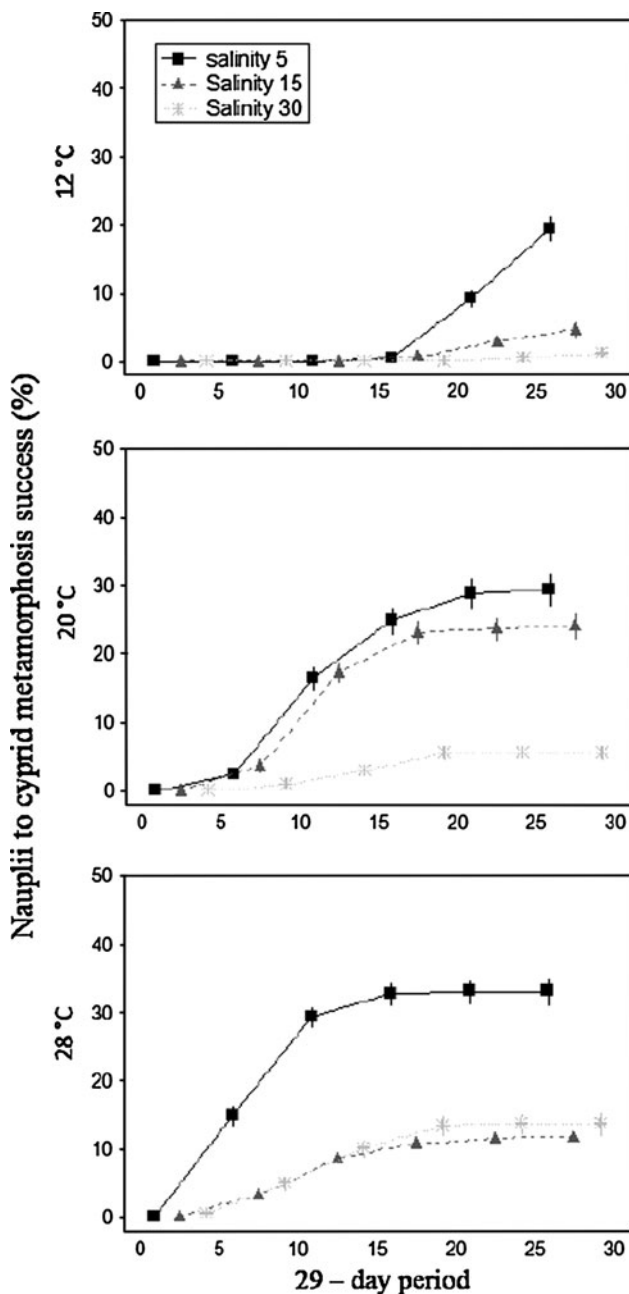
particularly low at 12°C where only under 5 psu it reached 20% by the end of the experiment (Fig. 2).

Survival of nauplii and cyprids (Exp. I) was affected by salinity but not by temperature, while there was no interaction between the two factors (Table 1b). Survival of nauplii decreased with increasing salinity under all temperature regimes with nauplii mortality ranging between 70% at 28°C/5 psu and almost 100% at 12°C/30 psu (Fig. 3). In contrast, cyprid mortality tended to be highest at 5 psu (except at the lowest temperature) and was low under all other treatment combinations (Fig. 3). The sum of nauplii and cyprid mortality always exceeded 70% and was particularly high under the combination of lowest temperature and highest salinity (Fig. 4a).

Among the survivors at the cyprid stage, settlement success (percent of the initial 20 nauplii settled) (Exp. I) was influenced by the main effects of temperature and salinity (Table 1c). Highest settlement occurred at the intermediate temperature and salinity combination, i.e., 20°C and 15 psu, while the lowest settlement was seen at 12°C—regardless of the salinity level (Fig. 4b). Final cyprid settlement success (percent of settlers relative to the number of surviving cyprids, Exp. I) tended to be lower at 12°C than in the two warmer treatments regardless of salinity (Fig. 5).

### Experiment II

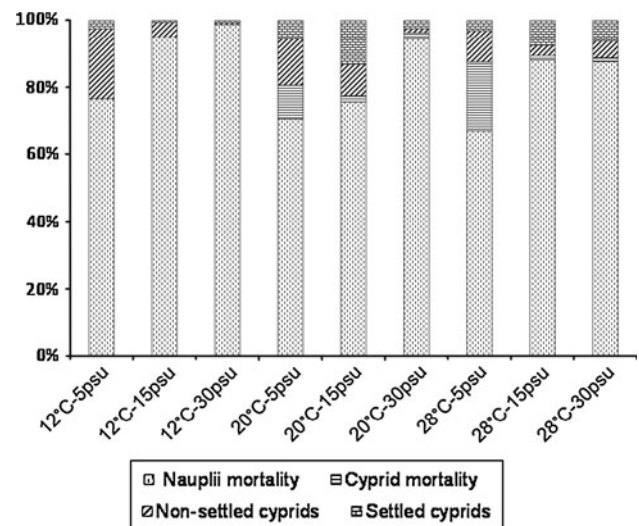
Settlement of cyprids without stress history (“naive” cyprids, Exp. II) was significantly affected by temperature and salinity, while no interaction between the two factors emerged (Table 2). Settlement success was high at 30 psu when combined with the low ( $\sim 90\%$ ) and the high ( $\sim 75\%$ ) temperature regime and was low when the intermediate ( $\sim 65\%$ ) temperature was applied (Fig. 6a). High and low temperatures decreased settlement success at 5 and 15 but



**Fig. 2** *Amphibalanus improvisus*. Success of nauplii metamorphosed to cyprids under different temperature and salinity regimes over a 29-day assay period. Each bar represents the mean ( $\pm$ SE) of eight replicates. Each replicate consisted of 20 nauplius larvae

not at 30 psu. Survival of the naive cyprids was high (>90%) under most factor combinations and only slightly reduced (~80%) at 28°C and 5 psu (Fig. 6b). The interaction between temperature and salinity on final cyprid survival was significant (ANOVA,  $F = 4.25$ ,  $df = 4$ ,  $p < 0.05$ ).

The two batches of cyprids used in Exp. I and II did not differ in their settlement success at no-stress conditions (20°C, 15 psu, Fig. 7,  $t$  test:  $t = 0.78$ ,  $df = 6$ ,  $p = 0.46$ ). In contrast, prestressed (at the nauplii stages) and naive



**Fig. 3** *Amphibalanus improvisus*. Performance of barnacle larvae after 4 weeks under different combinations of temperature and salinity (with prestressed larvae)

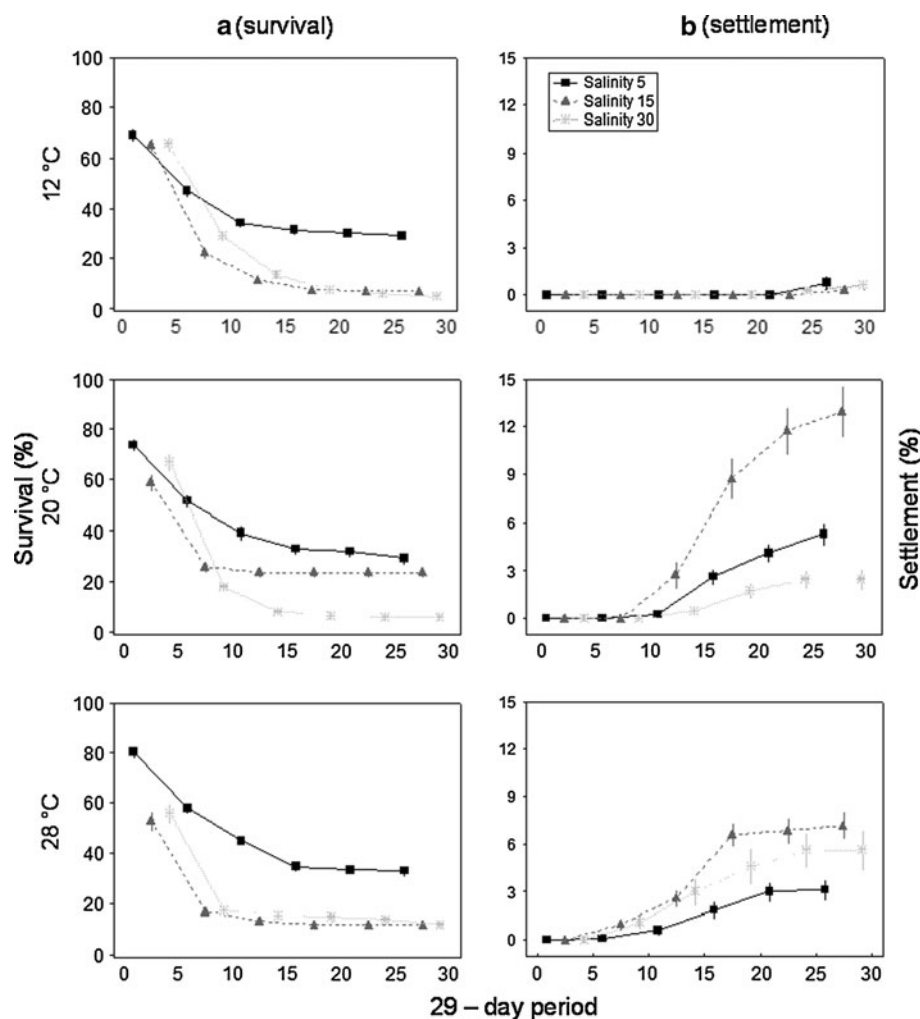
cyprids differed in their settlement at other stress conditions (Fig. 7).

## Discussion

Our results show that temperature and salinity have a strong potential to impact successful larval development of the barnacle *A. improvisus*. In our study, in several instances, the two factors interacted, i.e., the effect of one stressor depended on what level of the other stressor was present. Some factor combinations in our study were intentionally chosen to constitute suboptimal conditions, i.e., environmental stress, and we previously defined this as any setting of an environmental variable or several variables that reduces an organism's performance (Wahl et al. 2011). At the same time, we assumed that the environmental conditions during peak recruitment represent a scenario to which the population we investigated is adapted to, i.e., these conditions are optimal. Surprisingly, this was not always the case. In some instances, we observed that stress sensitivity of a given larval stage depended on the stress history experienced by earlier stages.

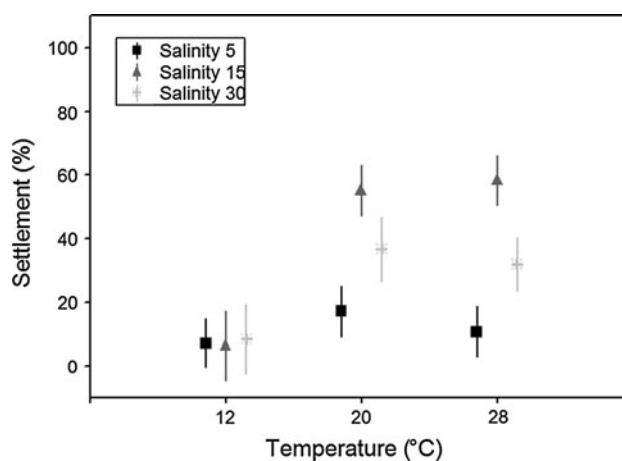
Total larval duration was shorter at higher temperatures. Similar results have previously been observed for *Amphibalanus amphitrite* (Anil et al. 1995, 2001; Qiu and Qian 1999), *B. trigonus* (Thiyagarajan et al. 2003a, b), *A. eburneus* (Scheltema and Williams 1982), *Elminius modestus* (Harms 1984, 1986), and *Semibalanus balanoides* (Harms 1984). Independently of salinity, larval duration was accelerated by 1.2 days per °C of warming ( $R^2 = 0.75$  and 0.96 for 15 and 5 psu, resp.). Salinity, in the range applied in our

**Fig. 4** *Amphibalanus improvisus*. Effect of temperature and salinity on cyprid survival **a** and settlement **b** in Exp. I (with prestressed larvae). Cyprid settlement was calculated as the percentage of all larvae in a replicate (20 nauplii) that settled. Survival has been accounted 24 h after the incubation of nauplii (day 1). Each bar represents the mean ( $\pm$ SE) of eight replicates. Each replicate consisted of 20 nauplius larvae



study, did not influence larval duration significantly. Inconsistently, Nasrolahi et al. (2006) found that increasing salinity led to a slight deceleration of larval development in *A. improvisus* in the Southern Caspian Sea population, where barnacles occur at an average salinity of 13 psu. In contrast, Anil and Kurian (1996) observed that total larval duration of *A. amphitrite*, which has a more marine distribution than *A. improvisus*, decreased with increasing salinity. The relatively low sensitivity toward salinity changes we observed in this species may arise from the fact that euryhaline barnacle species often have osmotic regulation by active ion transportation (Fyhn 1976; Gohad et al. 2009). *Amphibalanus improvisus* is widely distributed, inhabits warm temperate seas, and can be often found in areas of low (as the Baltic or Caspian Sea) or widely fluctuating salinities (Furman and Yule 1990).

The chance of survival of non-feeding cyprids depends upon the stored energy during the naupliar developmental history (Gaonkar and Anil 2010). Temperature and salinity stress may have an important effect on the amount of energy stored in nauplii by influencing the feeding pattern



**Fig. 5** *Amphibalanus improvisus*. Effect of temperature and salinity on final cyprid settlement in Exp. I (with prestressed larvae). Cyprid settlement includes percentage of settled cyprids from surviving cyprids. Each bar represents the mean ( $\pm$ SE) of eight replicates

(Anil and Kurian 1996) and/or by increasing their metabolic activity (Fraser 1989). This, as a consequence, influences the survival rate of cyprids. In this study, in contrast

**Table 2** *Amphibalanus improvisus*

Source	df	SS	MS	Pseudo- <i>F</i>	<i>p</i> (perm)	perms
Te	2	38.357	19.179	3.9185	0.0170	4,980
Sa	2	60.064	30.032	6.1361	0.0026	4,989
TexSa	4	30.97	7.7425	1.5819	0.1778	4,983
Res	27	132.15	4.8944			
Total	35	261.54				

Exp. II (naive cyprids) PERMANOVA testing of temperature and salinity on cyprid settlement

PERMANOVAs were based on the binomial dissimilarity measure. *p* values were obtained using 4,999 permutations of residuals under a reduced model

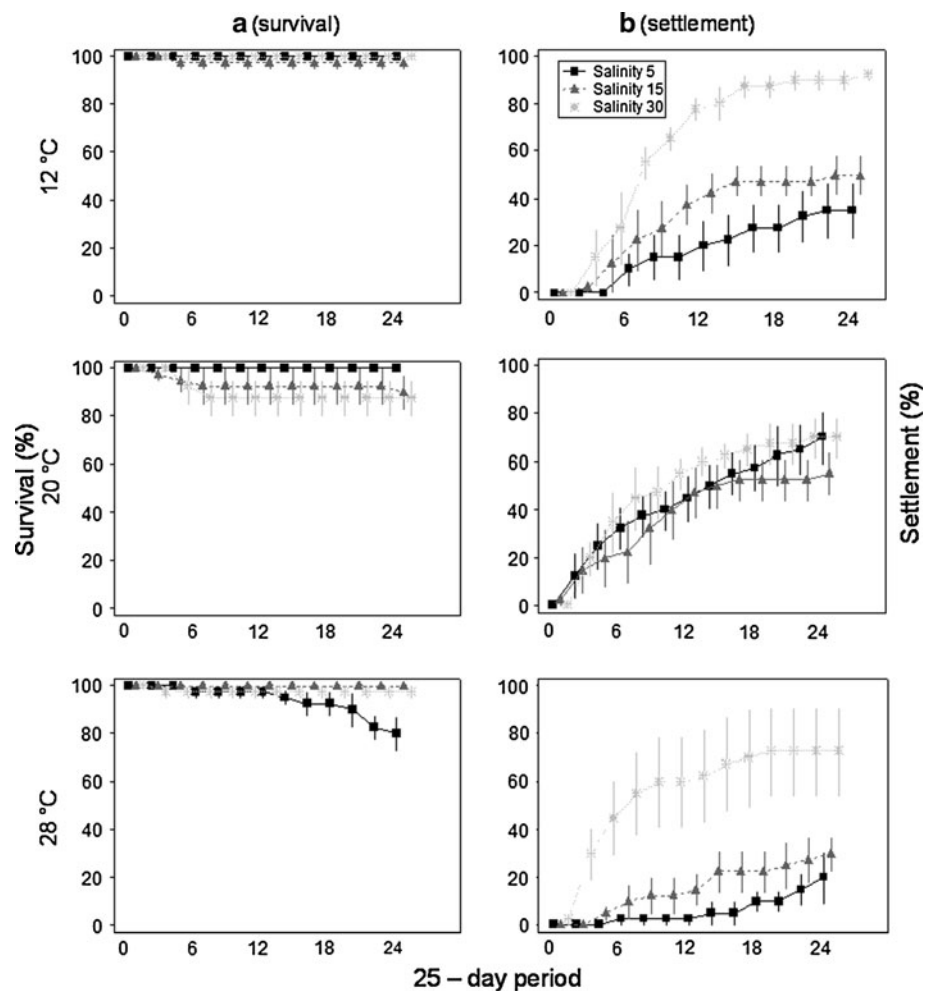
to larval duration, the rate of survival to the cyprid stage at all temperatures was highest at 5 psu. These results are similar to those reported by Nasrolahi et al. (2006) for the same species in the Caspian Sea. Possibly, low salinity compensates to some extent for negative effects of temperature stress on metamorphosis success in our experiments.

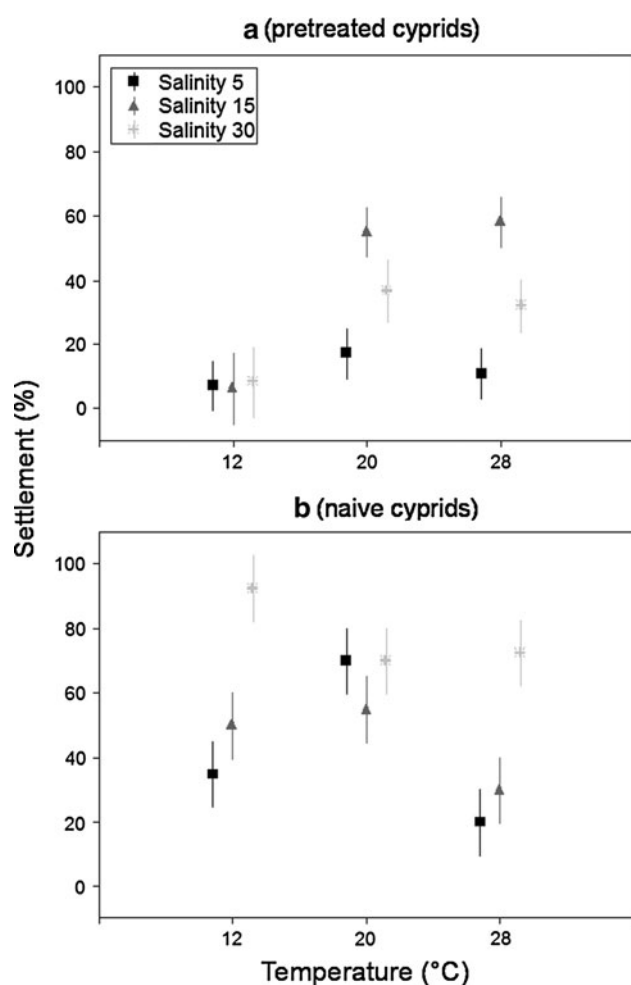
We found settlement to be almost zero at the lowest temperature (12°C), regardless of salinity, while survival at this

temperature was between 5 and 40% depending on salinity. However, we know that in the Western Baltic, nauplii, to some extent, hatch and survive at this and even lower temperatures as shown by Javidpour et al. (2010), who found barnacle nauplii in gut samples of *Mnemiopsis leidyi* in Kiel Fjord during most of winter. Surprisingly, though nauplii are obviously present in the water column at this time of the year, successful settlement is at an almost undetectable level between November and April (Wahl and Schütt, unpublished data; Thomsen et al. 2010). Various reasons for settlement failure of barnacle larvae during winter have been documented as, e.g., food constraint (Barnes and Barnes 1958; Thiagarajan et al. 2002a), low temperature (Anil et al. 1995), predation, starvation and misrouting (Crisp 1984), starvation in early naupliar stages (Lang and Marcy 1982), and finally the quality of released nauplii (Anil et al. 1995).

If the barnacle population we investigated was optimally adapted to the local conditions, we would have expected best performance (e.g., survival) among those individuals that experienced conditions prevailing during peak settlement in July and August (Thomsen et al. 2010), i.e., at

**Fig. 6** *Amphibalanus improvisus*. Effect of temperature and salinity on cyprid survival **a** and settlement **b** over a 25-day period in Exp. II (with naive cyprids). Day 0 shows settlement and survival after 24 h of incubation. Each bar represents the mean ( $\pm$ SE) of four replicates. Each replicate consisted of 10 cyprids





**Fig. 7** *Amphibalanus improvisus*. Final settlement under different temperature and salinity regimes in Exp. I (settled cyprids from surviving cyprids) **a** and in Exp. II **b**. In Exp. I cyprids were prestressed during their naupliar stage, while in Exp. II, cyprids were reared under habitat conditions. Each bar represents the mean ( $\pm$ SE) of eight replicates (Exp. I) or four replicates (Exp. II), respectively

about 20°C and 15 psu. However, larval duration was shortest at 28°C (regardless of salinity), metamorphosis success and overall survival (from start to end of Experiment I) was best at 5 psu (regardless of temperature). Only when we look at the proportion of settled cyprids, i.e., nauplii that completed their entire larval development and successfully transformed into settled, juvenile barnacles, then the “home” conditions of 20°C/15 psu proved the most beneficial (Exp. I, Figs. 3 and 4b). This is in line with Leppäkoski (1999) who showed that larval settlement in *A. improvisus* peaks at salinities around 15 psu.

Both increased temperature, via unfavorable de- or acceleration of metabolic activity (Pörtner and Farrell 2008; Neuheimer et al. 2011), and decreased salinity, due to physiological investment into osmoregulation (Pechenik et al. 2000), are potential stressors and therefore can reduce the amount of energy reserves available to non-feeding

cyprids. In our experiment, in naive cyprids (without stress history or carryover effects), settlement was lowest at a high temperature (28°C) and low salinity (5 psu) and highest at 30 psu, regardless of temperature. These results corroborate the findings of Thiyagarajan et al. (2003a) on *B. trigonus* in Hong Kong waters.

In our study, naive cyprids performed better under most salinity–temperature combinations than cyprids with a stress history (Fig. 7). The two experiments were performed on two separate batches of larvae, and therefore, differences between them might be due to differences between batches rather than differences in responses to the treatments. However, it is unlikely that these differences originate from using different larval batches of the same Kiel Fjord barnacle population in the two experiments. Comparing the two experiments, we could not detect any difference in settlement between those cyprids of the two batches the nauplii of which were raised under no-stress conditions (20°C/15 psu). On average, 20% more of naive cyprids settled than of pretreated cyprids (all nauplii treatments pooled). The combined effect of nauplii treatment (Exp. I) and cyprid treatment (Exp. II) is, however, complex because different larval stages exhibit different sensitivity patterns. For instance, naive cyprids settled best at 30 psu and least at 5 psu especially at intermediate and high temperatures (12 and 28°C), whereas the highest metamorphosis success of nauplii was found at 5 psu under all temperatures. When settlement success was expressed as percent of initial cyprid numbers in both experiments, only at the lowest temperature, carryover effects (i.e., higher sensitivity in prestressed as compared to naive cyprids) were detectable. Here, cyprids with a stress history at the naupliar stage reacted more sensitively to a given stress treatment than cyprids whose nauplii had been raised under no-stress conditions.

Osmotic stress experienced as found by Qiu and Qian (1999), in one life stage of a barnacle can be passed over to the next life stage. Corresponding to this, we saw that settlement of cyprids with a stress history (at the nauplius stage) was less than 15% of the initial number of nauplii, while in naive larvae (Exp. II), settlement and survival were much higher (relative to the initial number of cyprids, not nauplii). Such carryover effects could potentially work in two directions: either stress-induced selective mortality at the naupliar stages results in a preselection for stress resistance in the (surviving) cyprids population (composed of the more resistance genotypes) or stress conditions throughout the naupliar stages lead to unfit cyprids, which are low in energy, have a retarded development and exhibit higher mortality. Since we did not observe strong carryover effect, we assume that the two opposing effects compensated each other (except at the lowest temperature).

A temperature increase of more than 0.7°C during the last century has been documented for the Baltic Sea and a



further warming by up to 6°C is expected until 2100 (BACC 2008). Simultaneously, a decrease in salinity due to higher precipitation is predicted (BACC 2008). For Kiel Bight in the Western Baltic and during the season of peak barnacle settlement, this would correspond to a warming from 20 to 26°C and a desalination from 15 to a still unknown value between 14 and 8 psu (“up to –45%,” BACC 2008). If *A. improvisus* cannot adapt to these changes, both factors would additively or synergistically accelerate larval development (if food is not limiting), which could reduce larval mortality due to external factors such as food limitation or predation. Survival might slightly increase as suggested by the rates found for 20°C/15 psu (22%) and 28°C/5 psu (37%), respectively. Furthermore, our results suggest that settlement success of cyprids might decrease ~55% at 20°C/15 psu and ~10% at 28°C/5 psu. Thus, the beneficial effects of climate change on larval development and survival may be partially compensated by the detrimental effect on settlement success.

This scenario disregards the fact that *A. improvisus* undoubtedly has the potential to adapt to these changes to some, yet unexplored, extent, and it ignores further potential interactions of future salinity and temperature regimes with other changing environmental variables. Therefore, any prediction about climate change effects on species performance remains uncertain as long as we do not know about the compound effect of the whole suite of shifting environmental factors (temperature, salinity, nutrients,  $p\text{CO}_2$ , stratification, predation, parasitism, etc.) and the eco-evolutive potential, at all of their ontogenetic stages, of the affected species to adapt to these shifts (e.g., Reusch and Wood 2007; Wahl et al. 2011). Future studies on marine invertebrates under climate change scenarios should therefore focus more on the interactive effects of different environmental stressors considering all ontogenetic stages.

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