**Freshening of Great Barrier Reef waters is deleterious for larval crown-of-thorns starfish, implications that counter the terrestrial runoff hypothesis**

**Matthew Clements\*, Paulina Selvakumaraswamy, Dione Deaker, Maria Byrne**

**School of Life and Environmental Science, The University of Sydney, NSW 2006, Australia**

\* Corresponding author: matthew.clements@sydney.edu.au

Running page head: Salinity tolerance of *Acanthaster* sp. bipinnaria larvae

ABSTRACT: Outbreak populations of the crown-of-thorns starfish (COTS) cause widespread coral mortality. High nutrient river runoff generates phytoplankton blooms that potentially enhance larval food levels and success leading to outbreaks, a link posited by the terrestrial runoff hypothesis (TRH). Runoff plumes also lower salinity, although the parallel and potentially negative effects of freshening on COTS larvae, have not been considered. The impact of decreased salinity across a range of food levels on larval development and survival was investigated in context with the TRH. Larval survival and incidence of bipinnaria larvae with normal morphology in 6–7 salinities and 3 food treatments were quantified, at four time points, to generate salinity performance curves. Salinity was the major factor determining larval survival. At 24 hours of exposure the optimal salinity (*Sopt*) (≥90 % larval survival) was ~26–34 ‰ and the salinity with 50 % mortality (*LS50*) was 21.8 ‰. By 168 hours the *LS50* was 27.3 ‰, showing a narrowing of salinity tolerance over time. Low salinity impaired swimming resulting in larvae sinking at < 25–30 ‰. This was not due to halted ciliary beating and may be a mechanism to escape deleterious low salinity surface water. The sharp onset of deleterious effects at 22–25 ‰ is commensurate with salinity levels that larvae would experience in runoff plumes. Counter to the hypothesis of eutrophic enhancement of larval success and outbreaks, a paradigm that has driven management regulations for decades, runoff plumes are likely to be deleterious for COTS larvae.

KEY WORDS: River plumes, *Acanthaster* outbreaks, COTS, Coral reefs, Larval salinity performance, Survival, Swimming

1. **INTRODUCTION**

*Acanthaster*, a species complex of corallivorous sea stars called the crown-of-thorns (COTS) (Haszprunar & Spies 2014), are well known for their boom-and-bust population cycles (Uthicke et al. 2009, Deaker and Byrne 2022). Their notoriety derives from outbreak populations that cause widespread coral mortality throughout the Indo-Pacific (Endean 1973, Pearson 1981, Osborne et al. 2011, De’ath et al. 2012, Baird et al. 2013). Prior to recent back-to-back bleaching events (*see* Hughes et al. 2017,2019) the Pacific species of COTS was estimated to be responsible for 36–42 % of the coral mortality on the Great Barrier Reef (GBR) (Osborne et al. 2011, De’ath et al. 2012). Notably, when adult population density is low, their preferential foraging behaviour promotes coral species diversity (Done & Potts 1992). However, as one of the most efficient coral predators, large aggregations can contribute to the loss of entire coral colonies and whole reef scapes with severe ecological consequences that can lead to a phase shift to an algal/rubble state (Pratchett et al. 2014).

Despite significant allocation of resources to COTS research, particularly for the GBR, the causes of COTS outbreaks are not understood, and several hypotheses have been proposed (Pratchett et al. 2014, 2017, Deaker & Byrne 2022). As characteristic of planktotrophic larvae, COTS larvae develop faster at high food (phytoplankton) levels (Fabricius et al. 2010), up to a limit (Wolfe et al. 2015). The terrestrial runoff hypothesis (TRH) posits that phytoplankton blooms generated by high nutrient river discharge enhance larval survival and success, thereby driving outbreaks (Birkeland 1982). Nutrient replete larvae convey beneficial carryover benefits to enhance the success of the early benthic stage post larvae (e.g. Byrne et al. 2008). On the GBR, strong larval retention on reefs where the influence of river runoff/enhanced nutrients is high is suggested to link with COTS outbreaks (Wooldridge & Brodie 2015). However, despite predictions from the TRH, the temporal link between runoff events and subsequent COTS outbreaks has not been observed (Pratchett et al. 2014). This may be due to the ability of the juveniles to delay their transition to the adult stage for many years and build up as a reserve population in the reef infrastructure (juvenile in waiting hypothesis), confounding the direct links between larval cohorts and the appearance of adults (Deaker et al. 2020,2021). In addition, COTS larvae develop well under naturally low phytoplankton reef conditions (larval resilience hypothesis) (Olson 1987, Wolfe et al. 2015, 2017).

Terrestrial runoff causes significant freshening of GBR waters with low salinity (24–32 ‰) plumes inundating reefs for weeks, extending to depths of at least 18–20 m (Devlin et al. 2001, Brodie et al. 2005, Schroeder et al. 2012, Howley et al. 2018, eReefs: <http://www.ereefs.org.au/>). The historic impact of runoff is recorded in coral skeletons as an inverse relationship between salinity stress and coral calcification (D’Olivo et al. 2013). Moderate exposure to low salinity or long-term exposure to even slightly reduced salinity can result in coral bleaching and mortality (Van Woesik et al. 1995, Kerswell & Jones 2003). While river plumes most commonly impact near-shore reefs (20–30 km from the coast), they can extend for long distances (>100 km) to impact offshore reefs (King et al. 2000, Devlin et al. 2001, Devlin & Schaffelke 2009, eReefs: <http://www.ereefs.org.au/>). Flood events also coincide with the time that COTS larvae are in the plankton (November-April) and plumes of low salinity (26–30 ‰, to 5+ m depth) commonly impact the ‘initiation area’, the mid-shelf reefs considered to be source of larvae for COTS outbreaks (Wooldridge & Brodie 2015, eReefs: <http://www.ereefs.org.au/>).

Echinoderm larvae are well-known to have limited tolerance to low salinity with sea star larvae negatively impacted at salinities ≤ 30 ‰ (Roller & Stickle 1985, Stickle & Diehl 1987, Russel 2013), although larvae of some coastal species can tolerate ~20 ‰ (Bashevkin et al. 2016). There are several studies of the impact of decreased salinity on the development of COTS (Henderson 1969, Lucas 1973, Allen et al. 2017). The embryos tolerate low salinity to ~21 ‰ (Allen et al. 2017, Caballes et al. 2017). In a two-day exposure, bipinnaria larvae also tolerated 21 ‰ (Henderson 1969) and Lucas (1973) noted that 26 ‰ is the limit for successful metamorphosis. There are also reports that early development in COTS is faster in low salinity (30 ‰) (Henderson 1969, Lucas 1973). Environmental DNA data show that COTS larvae are broadly distributed in GBR surface waters (Uthicke et al. 2015) including at locations where decreased salinity occurs at levels known to be deleterious to sea star larvae.

It is important to understand how COTS larvae are impacted by environmental freshening in context with the TRH, because this hypothesis has driven management policies and regulations for decades with the assumption that curbing nutrients will also curb COTS outbreaks (Westcott et al. 2020). While riverine nutrient pollution control frameworks (e.g. DAWE 2021), are crucial for reef health, their connection to curbing COTS outbreaks is equivocal (Pratchett et al. 2014, Wolfe et al. 2017) and we have a poor understanding as to how COTS larvae respond to low salinity levels across the range that occurs in runoff events.

We investigated the impact of decreased salinity at levels that may be encountered in a GBR runoff event on the survival and normal development of the bipinnaria larvae of COTS and how the responses might be modulated by food levels. The larval swimming response to low salinity was also investigated. Salinity performance curves (SPC) were constructed for the larvae over time (24–168 hours of exposure), emulating runoff events that impact surface waters for days including around initiation area reefs (Pratchett et al. 2014). The SPCs were used to determine the salinity optimum (*Sopt*, salinity range ≥ 90 %) and the lethal salinity (*LS50*), with 50 % mortality.

As documented for the larvae of many sea star species (Stickle & Diehl 1987, Russel 2013, Bashevkin et al. 2016), it was hypothesised that low salinity would have negative effects on the bipinnaria larvae of COTS with reduced survival and altered swimming. Based on studies of developmental salinity tolerance in sea stars (Stickle & Diehl 1987, Russel 2013) we predicted that the tipping point for deleterious effects for COTS bipinnaria would be approximately 25 ‰. It was also predicted that increased phytoplankton food, including at densities that promote COTS larval success(Wolfe et al. 2017), may buffer the negative impacts of low salinity. As echinoderm larvae can actively avoid low salinity water (Metaxas & Young 1998a, 1998b), it was hypothesised that low salinity would reduce swimming resulting in a sinking response, potentially as a mechanism to evade deleterious surface conditions. In addressing the knowledge gap with respect to larval salinity tolerance we consider the implications for understanding the drivers of COTS outbreaks and management actions for the Great Barrier Reef, an ecosystem threatened by cumulative stressors from climate change in addition to COTS (Osborne et al. 2011, De’ath et al. 2012, Hughes et al. 2017, Kroon et al. 2020, DAWE 2021).

## MATERIALS & METHODS

* 1. **Spawning and rearing**

*Acanthaster sp*. were collected by the COTS control divers from the Northern Great Barrier Reef in November 2020 and shipped to Sydney by an aquarium supply company (Cairns Marine). The taxonomy of the pacific species of *Acanthaster* is uncertain (Haszprunar & Spies 2014) and so we refer to the species on the GBR as *Acanthaster* sp. or COTS. The sea stars were maintained at the Sydney Institute of Marine Science (SIMS) in flow-through aquaria at 26–27 **°**C, the temperature of their habitat (<http://www.ereefs.org.au/>).

To obtain gametes, a portion of the gonads was removed through a small incision at the base of the arms. The males and females chosen for use were highly fecund with gonads oozing gametes. For each of two fertilisations, gametes from two to three males and two females were used. The use of multiple parents for each fertilisation was used to emulate a synchronised spawning event, as occurs in nature (Babcock and Mundy 1992) and so the samples placed in the experimental vials (see below) would be different mixes of genotypes. Testes were placed in a tube and stored on ice until use. Ovaries were rinsed gently in 1.0 µm filtered sea water (FSW) and then placed in a dish of 10-5 M 1-methyl-adenine (1-MA) in FSW to induce ovulation. After ~40 minutes, the eggs were rinsed in FSW and examined with a stereomicroscope to confirm egg quality. The eggs were then placed in a 5 L beaker of FSW at 27 ºC﮿ The sperm solution was created by macerating testes and diluting them in FSW to induce sperm swimming. An equal mix of sperm from the two males was then added to fertilise the eggs (sperm concentration 105–106 sperm ml-1, as determined using a haemocytometer). Fertilisation, indicated by the presence of a fertilisation envelope, was confirmed to be > 90 %. Fertilised eggs were then rinsed in FSW to remove excess sperm. After 20 minutes the water was reduced by gentle reverse filtration and the water was renewed and the culture was split into several 5 L beakers of FSW until they hatched as gastrulae (~24 hours post fertilisation, hpf). The gastrulae were decanted into 2 L beakers and the water exchanged to achieve a density of ~3 embryos ml-1.

The embryos and larvae were reared at 27 °C (± 0.5 °C, n = 24) and 34 ‰ salinity (± 0.05 ‰, n = 24) in a constant temperature room (~27 °C). These parameters were checked using a WTW 2FD460 Multi 3420 MultiMeter conductivity sensor (Geotech Environmental). When they reached the bipinnaria stage, with a fully developed digestive tract at ~48 hpf (confirmed microscopically), feeding commenced for the cultures used for the fed treatments. These larvae were fed the tropical microalgae *Proteomonas sulcata* (20,000 cells mL-1) every 2 days after each water change. This food level is optimal for COTS larvae (Wolfe et al. 2017). Algal cell counts were determined using a haemocytometer. A parallel set of larval cultures were not fed. The larval cultures were gently aerated to maintain ~100 % (± 0.2 %, n = 10) dissolved oxygen and gentle agitation, measured with a 2FD460 Multi 3420 MultiMeter DO sensor (Geotech Environmental). The water was renewed every 2–3 days with water at temperature. This involved half water changes by reverse filtration. The beakers were also changed regularly to reduce the build-up of biofilm.

### **Experimental treatments**

Seven salinity treatments were used (control-34, 30, 25, 22, 19, 17, 15 ‰), levels commensurate with runoff plumes on the GBR (<http://www.ereefs.org.au/>) and extreme low salinities to identify lethal effects. To make the decreased salinity treatments, FSW was diluted with deionised water. The treatments were all at 27 °C (±0.5 °C, n = 8), ~100 % dissolved O2 (±0.2 %, n = 8) and pH 8.18-8.05. These parameters were checked with the 2FD460 Multi 3420 MultiMeter conductivity, DO and pH sensors (Geotech Environmental).

On day 7 post fertilisation (pf), prior to use of larvae in the salinity treatments, the fed cultures had their water renewed but they were not fed.

For salinity experiments, 10 larvae (10 days pf) were transferred to a small mesh basket in a dish (5 ml) of experimental water under a microscope with three changes of water and transferred into new dishes before being placed in the experimental vials (30 ml) with water at the same salinity level. This was done to ensure that the salinity in the experiment was not changed by pipetting of larvae as even small drops of control salinity water altered the salinity. Larvae that had never been fed were used for the no food treatment. Those from the fed cultures that had been starved for 3 days were randomly assigned to low (10,000 cells mL-1) and high (20 000 cells mL-1) food treatments. Vials containing larvae from the two fertilisations were placed randomly in the salinity treatments and were not monitored separately. There were 3–6 replicate vials randomly allocated to each salinity and feeding treatment (Table A1,A2), although 15 ‰ was not examined for the no food bipinnaria.

The vials (30 mL) were placed in a water bath maintained at ~27 °C. The larvae were examined after 24, 48, 96, and 168 hours of exposure. This timing depended on survival with some treatments having a shorter duration. Vial and water changes were made every 2 days. The larvae were examined microscopically prior to water change and scored for normal morphology and the number alive as indicated by motility.

### **Impact of salinity on larval swimming and position in the water column**

Larvae (n=5, 10 dpf) were placed in 5 mL vials with 5 salinity levels (19, 22, 25, 30, 34 ‰) in a temperature-controlled room (~27 °C). Each salinity treatment had 3 replicates. The location of the larvae in the water column in the vial was determined visually at 15, 30 and 60 minutes using a magnifying glass. By 15 minutes the larvae had settled into a position and the number of larvae swimming in the water column was scored. The percentage of larva swimming in the water column was determined. The larvae were allowed to settle after 15 minutes, and the 30 and 60-minute point was deemed to indicate the response of larvae in a plume. To check if larval sinking was due to cessation of ciliary beat, larvae (n = 5) that had sunk to the bottom of the vial in the 25 ‰ treatments at 60 minutes of exposure were examined microscopically (Olympus BX60).

### **Statistical analyses**

Data on the percentage of larval survival and normal morphology in the salinity and food treatments over time were plotted in box-and-whisker plots. We assessed the probability of normal morphology (1 = normal, 0 = non-normal) and survival (1 = survived, 0 = perished) occurring in COTS larvae using repeated measures generalised linear mixed-effect models (GLMM) with binomial error and logit link functions. Chi-squared tests on model residuals validated the model to be appropriately dispersed. Salinity, phytoplankton food level and hour of exposure were modelled as fixed effects; replicate was modelled as a random effect. GLMMs were fit with maximum likelihood estimates and nAGQ approximation using the glmer function (library: lme4) in R (R Core Team 2021). All combinations of fixed effects, including their interactions, were considered for each response variable and type II Walds Chi-Squared tests were used to select the best model fit of the data and to assess the significance of the fixed effects. Plots of significant GLMM model terms were constructed with 95 % confidence intervals (CI) to show salinity performance curves (SPCs) with respect to normal development and survival. We then determined the salinity optimum (*Sopt*: the salinity range with predicted survival ≥ 90 %) and the upper lethal salinity (*LS50*: the salinity with predicted mortality = 50 %) at each hour of exposure (24, 48, 96 and 168 hours) using binomial distributed generalised linear models (GLM) and logit link functions. Salinity was included in the models as a fixed effect on larval survival (1 = survived, 0 = perished). The dose.p function (library: MASS) in R (R Core Team 2021) was used to predict the salinity range/level at which the *Sopt*and *LS50*values occur. Model fits and their compliance with statistical assumptions were assessed using visual inspection of residual vs fitted plots.

Data for the percentage of larvae swimming in the water column were analysed using a two-way analysis of variance (ANOVA) with duration of exposure (2 levels: 30 and 60 minutes) and salinity (5 levels: 19, 22, 25, 30, 34 ‰) as fixed factors. The data were arcsine transformed and homogeneity of variance was confirmed (Levene’s test). Normality was not achieved as determined by visual inspection of Q-Q plots, but as ANOVA is robust to such violation with equal sample sizes (Quinn & Keough 2002) this analysis was used. Tukey HSD post hoc tests for significant pairwise comparisons were computed between levels of salinity. The significance level for all tests was p < 0.05. All analyses were performed in R (v1.2.1335) using Rstudio (R Core Team 2021).

## RESULTS

* 1. **Impacts of salinity on larvae over time**

Overall, for the percentages of bipinnaria with a normal morphology and survival, salinity levels < 22–25 ‰ were deleterious and the salinity performance curves (SPCs) show the negative cumulative effects of reduced salinity over time (Fig.1-3; Table A1,A2).

Both salinity and time had a significant influence on the probability of the occurrence of normal bipinnaria (Salinity: 𝜒2 = 93.43, df = 5, p **<** 0.001**;** Time: 𝜒2 = 207.59, df = 3, p **<** 0.001, Table 1). The three-way interaction and all two-way interactions between salinity, food and time, and the food factor on its own, did not significantly influence the model fit to the normal bipinnaria data (Table 1). The performance curves (Fig. 3A) show 100 % mortality in the 17–19 ‰ treatments and a higher percentage of normal bipinnaria with increased salinity. The sharp decrease in the percentage of normal bipinnaria at 22–25 ‰ indicated that this salinity range approximated the tolerance tipping point (Fig. 3A).

Both salinity and time had a significant influence on the probability of larval survival (Salinity: 𝜒2 = 99.45, df = 5, p **<** 0.001**;** Time: 𝜒2 = 194.88, df = 3, p **<** 0.001, Table 2). The three-way interaction and all two-way interactions between salinity, food, and time, and the food factor on its own, did not significantly influence the model fit (Table 2). There was a sharp decrease in larval survival at 22–25 ‰ (Fig. 3B).

The decline in survival over time is shown by the increase of the *LS50* and lower salinity limit of the *Sopt* (Fig. 3B). The *Sopt* range decreased from 25.9–34 ‰ at 24 h of exposure to 32.2–34 ‰ at 96 h of exposure (Table 3). After one week in treatments (168 h of exposure), survival in the controls was below 90 % and so the *Sopt* range was not determined. The *LS50*, (Fig. 3B) increased from 21.8 ‰ at 24 h of exposure to 27.32 ‰ at 168 h of exposure (Fig. 3B, Table 3).

### **The effect of salinity on the number of larvae swimming in the water column**

Salinity significantly affected the percentage of larvae swimming in the water column (ANOVA F4,20 = 63.2, p< 0.001, Table 4). Duration of exposure (30 and 60 minutes) and the interaction between exposure duration and salinity did not significantly affect the percentage of larvae swimming (Table 4). The highest percentage (≥ 86.67 %) of larvae swimming in the water column occurred in the 30–34 ‰ treatments (Fig. 4, Table A3). At 30 min of exposure there was a decrease in the percentage of swimming larvae at all salinities below controls with all the larvae at the bottom of the vials in the 19 and 22 % treatments (Tukey’s HSD: 19 = 22 < 25 < 30 = 34 ‰). From 30 to 60 min of exposure, there was a further decrease in the percentage of swimming larvae for those exposed to 25 ‰ only, however this was not statistically significant (Table 4). At 25 ‰ the percentage of swimming larvae decreased from 46.67 % at 15 min of exposure to 13.33 % at 60 min of exposure (Fig. 4, Table A3). It appeared that 25 ‰ approximated the tipping point salinity for a marked decrease in the number of larvae swimming in the water column (Fig. 4). Microscopic examination of larvae in the 25 ‰ treatment that had sunk to the bottom of the vial after 60 min of exposure revealed that the cilia were still beating. Larvae in the low salinity (19–25 ‰) treatments examined after 24 h of exposure had shorter lateral processes and had a swollen appearance (Figure 5).

### **DISCUSSION**

We determined the response of the larvae of *Acanthaster sp.* to decreased salinity in the context of the terrestrial runoff hypothesis and conditions likely to be experienced in runoff events. As predicted, decreased salinity had a strong negative impact on the larvae, regardless of food level, at salinity levels that occur where and when COTS larvae are in the plankton.

After 24 and 168 h of exposure, the *LS50*’s for the bipinnaria were 21.8 and 27.32 ‰, respectively, results similar to the negative effects reported for these larvae at 48 (21 ‰) and 92 (26 ‰) h of exposure (Henderson 1969; Lucas 1973). The *Sopt* estimate suggests that the bipinnaria would have decreased performance with respect to normal development and mortality in pulses of salinities < 29 ‰ after 48 h of exposure. The *LS50*’s showed that salinity tolerance reduced by 1 salinity unit over the first two days and by 5.52 units by the end of the experiment at 168 h of exposure. Thus, the cumulative effects of osmotic stress in runoff plumes that can last for days and sometimes weeks (Devlin et al. 2001, eReefs: <http://www.ereefs.org.au/>) would be deleterious for COTS larvae. Decreasing stress tolerance with time is characteristic for marine invertebrate larvae (Pechenik 2018).

In comparison with larval tolerance levels, fertilisation and embryonic development in COTS are more sensitive to low salinity, as typical of marine invertebrates (Madrones-Ladja 2002, Pineda et al. 2012). Fertilisation success declines at 23–25 ‰ and embryos delay hatching and have high abnormality at < 29 ‰ and < 27 ‰, respectively (Allen et al. 2017). In acute 2 h tests, embryo abnormality was observed at 26–28 ‰ (Caballes et al. 2017). However, as the gametes and embryos of COTS are likely to remain near the sea floor, they would not be expected to encounter low salinity surface waters.

That COTS larvae develop faster in low salinity-warm conditions (30 ‰/~28 °C) with high survival (Lucas 1973) is taken as evidence to support the TRH (Brodie et al. 2005). However, these larvae had total metamorphic failure (Lucas 1973) and so would not recruit to form an outbreak population. Moreover, 30 ‰ did not have a negative effect on the larvae investigated here until 4 days of exposure. It is likely that the faster growth recorded by Lucas (1973) was due to warming (2 ºC increase).

The number of larvae swimming in the water column was markedly reduced at salinities ≤ 25–30 ‰ resulting in the sinking response. No larvae were swimming in the 19 ‰ and 22 ‰ treatments and the percentage of swimming bipinnaria decreased 3-fold in the 25 ‰ treatment over time. The continued ciliary beating activity in larva that had sunk to the bottom of the vials in this salinity indicated that ciliary movement no longer worked to maintain the larvae in the water column and that the mechanics of the beat had changed.

The swollen appearance and shorter arms on the larvae in low salinity is likely due to movement of water molecules into the larvae as they are osmoconformers (Russell 2013). Similarly, the bipinnaria of the sea star *Pisaster ochraceus* exposed to ~20 ‰ salinity for 1–3 weeks developed into wider larvae with shorter posterolateral arms, which had altered swimming behaviour (Pia et al. 2012, Bashevkin et al. 2016). Changes in larval shape and density alters the buoyancy, swimming behaviour, sinking rate, and passive vertical dispersal of echinoderm larvae in response to decreased salinity, influencing their position in the water column (Chia et al. 1983, Young 1995, Metaxas & Young 1998a, 1998b, Vazquez & Young 1996, Chan 2012, Pia et al. 2012, Bashevkin et al. 2016).

Echinoderm larvae have some ability to control their swimming and vertical position in the water column in response to low salinity and food (Metaxas & Young 1998a, 1998b, 1998c, Bashevkin et al. 2016). The sinking of COTS larvae may be an active behavioural response to osmotic stress to escape low salinity, as suggested for ascidian larvae that sink below the halocline (Vázquez & Young 1996). With distance from sources of river plumes, the halocline discontinuity layer occurs at increasingly shallower depths (eReefs: <http://www.ereefs.org.au/>) and so COTS larvae on mid-shelf reefs would not need to sink as far to escape deleterious surface salinities compared to larvae in waters closer to the coast. The sinking response may also allow COTS larvae to encounter a food pulse as many zooplankton species including sea urchin and sea star larvae aggregate at haloclines to avail of phytoplankton food that concentrates at these discontinuity layers (Metaxas & Young 1998a, 1998b, 1998c, Bashevkin et al. 2016). However, it is not known if COTS larvae can recover from osmotic shock as they sink to higher salinity water as well as their ability to behaviourally respond to haloclines and food concentrations. The position of COTS in the water column and their ability to modulate their vertical distribution and the potential influence of haloclines and phytoplankton accumulations on larval behaviour are not known (Pratchett et al. 2017). These are important to understand as these behaviours would influence larval dispersal.

* 1. **Implications for the terrestrial runoff hypothesis**

The TRH is based on the potentially beneficial response of COTS larvae to increased availability of phytoplankton food that accompany run off events (Birkeland 1982). So far, the covarying factor of freshening on COTS larvae has not been considered. Our data show that larval success also requires favourable salinity or non-deleterious short-lived low salinity pulses. On the GBR, COTS larvae are likely to experience salinity levels of 24–30 ‰ (and lower) in association with flood plumes (Devlin et al. 2001, Jones & Berkelmans 2014). River runoff plumes with salinity levels that cause high mortality to COTS larvae (26–30 ‰ by 96 h of exposure) can inundate initiation area reefs for days (Devlin et al. 2001; Devlin & Brodie 2005; Schroeder et al. 2012; eReefs: <http://www.ereefs.org.au/>). Strong larval retention combined with runoff nutrients is suggested to promote success of COTS larvae on these reefs (Wooldridge & Brodie 2015). However, our data suggest that the negative effects of decreased salinity as well as accompanying toxicants (Kroon et al. 2020) would outweigh the potential benefits of higher food.

Although it may be expected that the higher levels of nutrients recorded on inshore reefs during runoff would enhance larval success and settlement (Brodie et al. 2005), adult COTS are rarely observed on near shore reefs (AIMS 2015). Larval sensitivity to low salinity, suggests that if they encounter water with a salinity < 24–30 ‰ for 1-4 days, as occurs frequently on reefs within 20 km of the coast (eReefs: <http://www.ereefs.org.au/>), then high mortality would occur. These reefs can also experience very low salinity (to 6.5–8 ‰) in long durations to depths of 5–9 m (Van Woesik 1991, Jones & Berkelmans 2014, eReefs: <http://www.ereefs.org.au/>), conditions lethal for COTS larvae. Moreover, these conditions also have deleterious effects on the preferred coral prey of COTS (Van Woesik et al. 1995, Kerswell & Jones 2003, Keesing 2021).

Chl-*a* and ocean colour satellite data are used as proxies to estimate phytoplankton biomass in GBR waters (Devlin et al. 2013a, Brodie et al. 2017). Based on these remote measures, the highest chl-*a* occurs in water with 10–25/30 ‰ salinity (Devlin & Schaffelke 2009, Devlin et al. 2013a), freshening levels deleterious to COTS larvae. High concentrations of chl-*a* occur at low salinity-high salinity plume fronts (Devlin & Brodie. 2005, Brodie et al. 2017). While the chl-*a* levels generated by river plumes across a large spatial extent of the GBR are suggested to indicate the presence of optimal food availability for COTS larvae (Devlin & Brodie 2005, Devlin & Schaffelke 2009), how these data relate to phytoplankton species composition and salinity levels in locations where COTS larvae occur are not known. For instance, the euryhaline diatom *Skeletonema* occurs in low salinity plumes (Devlin et al. 2013b), but it is not known if they are accessible to COTS larvae with respect to salinity levels. Direct measurement of water samples shows decreasing phytoplankton at salinities > 25 ‰, indicating that favourable salinity and food may not coincide for COTS larvae (Howley et al. 2018).

While there are many laboratory studies of feeding in COTS larvae (e.g., Wolfe et al. 2015, Mellin et al. 2017), further knowledge is needed as to the selective feeding of COTS larvae and direct determination of phytoplankton species assemblages in situ (Brodie et al. 2017, Pratchett et al. 2017). These data are needed with focus on regions with favourable salinity for COTS larvae to assess the hypothesised link between nutrient enrichment and larval success and, importantly, to determine the veracity of chl-*a*/ocean colour as proxies to identify favourable conditions for COTS larvae. We do not know what salinity levels COTS larvae encounter in the plankton with respect to potential low salinity-high food scenarios. It is also not known if terrestrial runoff generates favourable phytoplankton conditions for larvae at surface plume fronts or at halocline discontinuities that the larvae may find as they sink to deeper water.

Relating COTS outbreaks to runoff events with respect to salinity and food conditions is complex due to the influence of regional oceanography, reef connectivity and the spatial patterns of outbreaks (Wooldridge & Brodie 2015). Our results show the importance to consider river runoff as a potential source of mortality for COTS larvae, especially as decreased salinity coincides with the increased nutrient conditions assumed to promote larval success and drive outbreaks. Understanding the low salinity conditions that COTS larvae encounter in nature now, and, potentially under future flood events are important in predicting outbreaks in the face of increased freshening and anthropogenic contamination of marine waters due to climate change (Howley et al. 2018, Kroon et al. 2020, IPCC 2021). While management regulation to reduce runoff of nutrients and other contaminants is crucial to protect the Great Barrier Reef, whether these actions address the wicked COTS problem (Deaker & Byrne 2022), remains to be determined.

*Data availability.* The data and code that support the findings of this study are available on request from the corresponding author.

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TABLES

Table 1. Type II Walds Chi-Squared test results on binomial-error distributed generalised linear mixed-effect models (GLMM) for model selection. GLMMs were used to predict the probability of normal morphology (proportional binomial response variable: 1 = normal, 0 = non-normal) in COTS bipinnaria larvae as a function of salinity level (Salinity), phytoplankton food level (Food), hour of exposure (Time) and their interactions (as indicated by asterisks). All models include the experimental unit (‘id’) as a random effect. The models performed are indicated in the left column. Model Terms define the parameters tested. Significant Wald Chi-squared statistics (Waldχ2) regard model parameters that significantly improve the model fit to the data and are indicated in bold (p ≤ 0.05), as indicated in the best fit model.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Model | Model Terms | Waldχ2 | df | p |
| Salinity\*Food\*Time+(1|id) | Salinity\*Food\*Time | 7.42 | 30 | 0.972 |
| (Salinity+Food+Time)2+(1|id) | Salinity\*Food | 14.54 | 10 | 0.15 |
| Salinity\*Time | 15.29 | 15 | 0.431 |
| Food\*Time | 5.83 | 6 | 0.443 |
| Salinity+Food+Time+(1|id) | Salinity  Food  Time | 96.07  2.6  204.09 | 5  2  3 | **< 0.001**  0.273  **< 0.001** |
| Salinity+Time+(1|id) | Salinity  Time | 98.6  210.31 | 5  3 | **< 0.001**  **< 0.001** |

Table 2.Type II Walds Chi-Squared test results on binomial-error distributed generalised linear mixed-effect models (GLMM) for model selection. GLMMs were used to predict the probability of survival (proportional survival response variable: 1 = survived, 0 = perished) in COTS bipinnaria larvae as a function of salinity level (Salinity), phytoplankton food level (Food), hour of exposure (Time) and their interactions (as indicated by asterisks). All models include the experimental unit (‘id’) as a random effect. The models performed are indicated in the left column. Model Terms define the parameters tested. Significant Wald Chi-Squared statistics (Waldχ2) regard model parameters that significantly improve the model fit to the data and are indicated in bold (p ≤ 0.05), as indicated in the best fit model.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Model | Model Terms | Waldχ2 | df | p |
| Salinity\*Food\*Time+(1|id) | Salinity\*Food\*Time | 7.06 | 30 | 1.000 |
| (Salinity+Food+Time)2+(1|id) | Salinity\*Food | 11.67 | 10 | 0.308 |
| Salinity\*Time | 15.47 | 15 | 0.418 |
| Food\*Time | 6.52 | 6 | 0.367 |
| Salinity+Food+Time+(1|id) | Salinity  Food  Time | 113.18  4.77  209.21 | 5  2  3 | **< 0.001**  0.092  **< 0.001** |
| Salinity+Time+(1|id) | Salinity  Time | 97.73  202.95 | 5  3 | **< 0.001**  **< 0.001** |

Table 3.Optimum salinity range (*Sopt*) with ≥ 90 % survival of bipinnaria at four exposure times (24–168 h) to six salinity treatments (17–34 ‰) and the upper lethal salinity limit (*LS50*) for 50 % survival. By day 7 (168 h) survival in the controls was below 90 % and so the *Sopt* range was not determined.

|  |  |  |
| --- | --- | --- |
| Exposure time | *LS50* | *Sopt* |
| 24 | 21.8 ‰ | 25.9-34 ‰ |
| 48 | 22.8 ‰ | 29.2-34 ‰ |
| 96 | 24.7 ‰ | 32.2-34 ‰ |
| 168 | 27.3 ‰ | – |

Table 4. Two-way ANOVA of data on the percentage of larvae swimming in the water column in 5 salinity treatments at 30 and 60 minutes exposure and Tukey’s HSD post-hoc test results.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Factor** | **SS** | **df** | **MS** | **F** | **P** | **Tukey’s HSD** |
| Salinity | 13.36 | 4 | 3.34 | 63.20 | < 0.001 | 19 = 22 < 25 < 30 = 34 ‰ |
| Duration of exposure | 0.04 | 1 | 0.04 | 0.80 | 0.381 |  |
| Salinity x duration of exposure | 0.17 | 4 | 0.04 | 0.80 | 0.537 |  |
| Residual | 1.06 | 20 | 0.05 |  |  |  |
| Total | 14.63 | 29 | 3.47 |  |  |  |

FIGURES

|  |
| --- |
|  |
| Diagram, schematic  Description automatically generated  Fig. 1. Unfed larvae reared in six salinity treatments over one week. (A) The percentage of normal larvae. (B) Percentage larval survival. The box plots show treatment medians and interquartile range; maximum and minimum. Means and standard errors are provided in Table A1 & A2. |

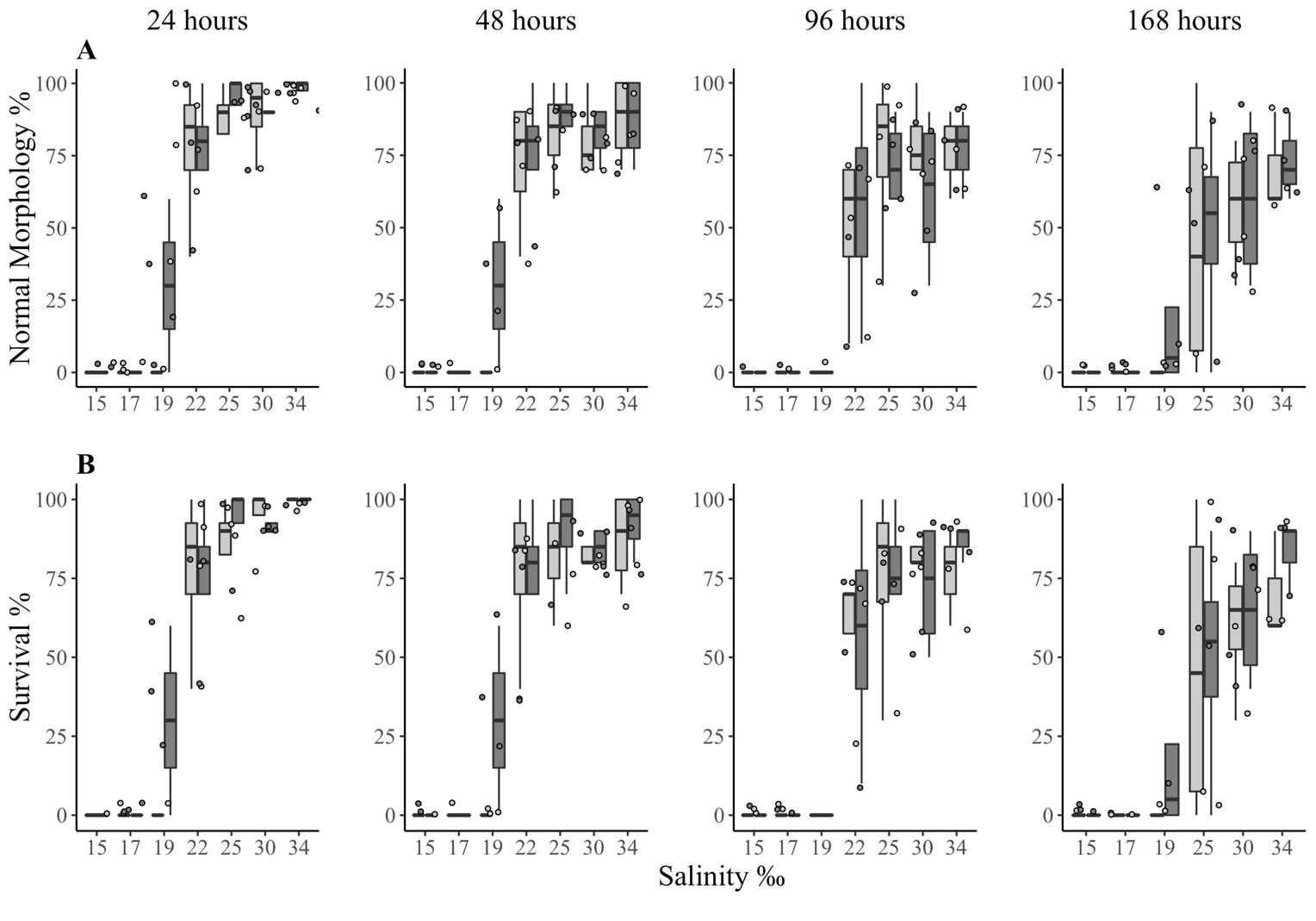


Fig. 2. Fed larvae in high (dark grey) and low (light grey) food treatments reared in seven salinity treatments over one week. (A) The percentage of normal larvae. (B) Percentage larval survival. The box plots show treatment medians and interquartile range; maximum and minimum. Means and standard errors are provided in Table A1 & A2.

|  |
| --- |
|  |
| |  | | --- | | Fig. 3. Bipinnaria salinity performance curves (SPC) for the probability of (A) normal larval morphology and (B) survival at four exposure times (24–168 hours). The best generalised linear mixed-effect model fit (Table 1,2) was used to generate the curves. The bars indicate 95 % confidence intervals (CI). CI’s that do not overlap indicate significant differences. Arrows indicate performance thresholds. Short arrows indicate the lower range salinity optimum (*Sopt*), and long arrows, the lethal salinity with 50 % mortality (*LS50*) (see Table 3). By day seven, survival in the controls was below 90 % and so the *Sopt* range was not determined. | |

|  |  |
| --- | --- |
| Fig. 4. A-C. Percentage of swimming bipinnaria larvae in five salinity treatments at 15, 30 and 60 minutes. The box plots show treatment medians and interquartile range; maximum and minimum. See Table A3 for means and standard errors.     |  | | --- | | Figure 5. The appearance of *Acanthaster* sp.bipinnaria larvae (12 days post fertilisation) at various salinity treatments after 24 hours of exposure. Note the swollen like appearance and reduction in larval body size in larvae exposed to salinity ≤ 25 ‰ versus the control 34 ‰. | |

Appendices

Table A1. Percentage of bipinnaria with normal morphology in salinity and food treatments at 24, 48, 96, and 168 h of exposure. At 15 ‰ (n = 4) and 17 ‰ (n = 3-4) mortality was 100%. Values are mean (± SE, n).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | | Salinity Treatment (‰) | | | | |
| Hours of exposure | Food  density | 19 | 22 | 25 | 30 | 34 |
| 24 | NF | 10 (5.8, 3) | 46 (14.4, 5) | 90 (5.5, 5) | 90 (7.7, ) | 98.3 (1.7,6) |
|  | LF | 0 (0, 4) | 77.5 (13.1, 4) | 85 (8.7, 4) | 90 (7.1, 4) | 100 (0, 4) |
|  | HF | 30 (12.9, 4) | 75 (12.6, 4) | 92.5 (7.5, 4) | 90 (0, 4) | 97.5 (2.5, 4) |
| 48 | NF | 0 (0, 3) | 32 (17.7, 5) | 76 (12.5, 5) | 88 (7.3, 5) | 95 (3.4, 6) |
|  | LF | 0 (0, 4) | 72.5 (11.8, 4) | 82.5 (8.5, 4) | 80 (7.1, 4) | 87.5 (7.5, 4) |
|  | HF | 30 (12.9, 4) | 75 (12.6, 4) | 87.5 (6.3, 4) | 82.5 (4.8, 4) | 87.5 (7.5, 4) |
| 96 | NF | 0 (0, 3) | 14 (9.8, 5) | 54 (15, 5) | 82 (5.8, 5) | 87.5 (2.5, 4) |
|  | LF | 0 (0, 4) | 50 (14.1, 4) | 75 (15.5, 4) | 82.5 (6.3, 4) | 76.7 (8.8, 3) |
|  | HF | 17.5 (14.4, 4) | 57.5 (18.9, 4) | 72.5 (7.5, 4) | 62.5 (13.8, 4) | 76.7 (8.8, 3) |
| 168 | NF | 0 (0, 3) | 12 (2, 5) | 34 (18.71, 5) | 70 (14.97, 5) | 87.5 (2.5, 4) |
|  | LF | 0 (0, 4) | 30 (15.81, 4) | 45 (23.98, 4) | 57.5 (11.09, 4) | 70 (10, 3) |
|  | HF | 10 (7.07, 4) | 37.5 (19.31, 4) | 50 (13.78, 4) | 60 (11.90, 4) | 73.3 (6.67, 3) |

Table A2. Percentage of bipinnaria survival in salinity and food treatments at 24, 48, 96, and 168 h of exposure. At 15 ‰ (n = 4) and 17 ‰ (n = 3-4) mortality was 100%. Values are mean (± SE, n).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | | Salinity Treatment (‰) | | | | | |
| Hours of  exposure | Food density | 19 | 22 | 25 | 30 | 34 |
| 24 | NF | 10 (5.8, 3) | 46 (14.4, 5) | 92 (3.7, 5) | 90 (7.7, 5) | 98.3 (1.7, 6) |
|  | LF | 0 (0, 4) | 77.5 (13.1, 4) | 85 (8.7, 4) | 95 (5, 4) | 100 (0, 4) |
|  | HF | 30 (12.9, 4) | 75 (12.6, 4) | 92.5 (7.5, 4) | 92.5 (2.5, 4) | 100 (0, 4) |
| 48 | NF | 0 (0, 3) | 32 (17.7, 5) | 80 (8.9, 5) | 88 (7.3, 5) | 95 (3.4, 6) |
|  | LF | 0 (0, 4) | 77.5 (13.1, 4) | 82.5 (8.5, 4) | 85 (5, 4) | 87.5 (7.5, 4) |
|  | HF | 30 (12.9, 4) | 75 (12.6, 4) | 90 (7.1, 4) | 85 (2.9, 4) | 92.5 (4.8, 4) |
| 96 | NF | 0 (0, 3) | 16 (11.7, 5) | 54 (15, 5) | 82 (5.8, 5) | 87.5 (2.5, n=4) |
|  | LF | 0 (0, 4) | 57.5 (12.5, 4) | 75 (15.5, 4) | 85 (5, 4) | 76.7 (8.8, n=3) |
|  | HF | 17.5 (14.4, 4) | 57.5 (18.9, 4) | 80 (7.1, 4) | 72.5 (10.3, 4) | 86.7 (3.3, n=3) |
| 168 | NF | 0 (0, 3) | 14 (7.5, 5) | 36 (15.0, 5) | 70 (13.0, 5) | 87.5 (2.5, n=4) |
|  | LF | 0 (0, 4) | 30 (15.8, 4) | 47.5 (25, 4) | 60 (10.8, 4) | 70 (10, n=3) |
|  | HF | 10 (7.1, 4) | * 1. (19.3, 4) | 50 (18.7, 4) | 65 (11.9, 4) | 83.3 (6.7, n=3) |

Table A3. The percentage of larvae that were swimming in five salinity levels at three time points. Values are means (± SE), n = 3.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Salinity (‰) | | | | |
| Time (min) | 19 | 22 | 25 | 30 | 34 |
| 15 | 0 (0) | 0 (0) | 46.67 (13.33) | 100 (0) | 100 (0) |
| 30 | 0 (0) | 0 (0) | 33.33 (13.33) | 86.67 (13.33) | 100 (0) |
| 60 | 0 (0) | 0 (0) | 13.33 (13.33) | 86.67 (13.33) | 100 (0) |