RESEARCH ARTICLE

Suspended sediment prolongs larval development in a coral reef fish

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ABSTRACT
Increasing sediment input into coastal environments is having a profound influence on shallow marine habitats and associated species. Coral reef ecosystems appear to be particularly sensitive, with increased sediment deposition and re-suspension being associated with declines in the abundance and diversity of coral reef fishes. While recent research has demonstrated that suspended sediment can have negative impacts on post-settlement coral reef fishes, its effect on larval development has not been investigated. In this study, we tested the effects of different levels of suspended sediment on larval growth and development time in Amphiprion percula, a coral reef damselfish. Larvae were subjected to four experimental concentrations of suspended sediment spanning the range found around coastal coral reefs (0–45 mg l⁻¹). Larval duration was significantly longer in all sediment treatments (12 days) compared with the average larval duration in the control treatment (11 days). Approximately 75% of the fish in the control had settled by day 11, compared with only 40–46% among the sediment treatments. In the highest sediment treatment, some individuals had a larval duration twice that of the median duration in the control treatment. Unexpectedly, in the low sediment treatment, fish at settlement were significantly longer and heavier compared with fish in the other treatments, suggesting delayed development. A sediment-induced extension of the pelagic larval stage could significantly reduce numbers of larvae competent to settle and, in turn, have major effects on adult population dynamics.

KEY WORDS: Turbidity, Larvae, Metamorphosis

INTRODUCTION
The development and modification of coastal zones around the globe have led to a reduction in the water quality of inshore marine ecosystems (Cloern, 2001; Tilman et al., 2001; Boesch, 2002; Duarte, 2002). Among these, coral reefs are especially susceptible to increases in nutrient levels, sediment loads and pollutants from land-based sources (Sebens, 1994; Fabricius, 2011; Kroon et al., 2012). Exposure to reduced water quality has been linked to declines in the abundance of coral reef organisms and shifts in species composition (Letourneur et al., 1998; Mallela et al., 2007; De’ath et al., 2006). If high turbidity does ultimately reduce feeding efficiency in coral reef fish larvae, then growth and body condition would be expected to decline with increasing sediment, while pelagic larval duration (PLD) should increase as development slows (e.g. Green and McCormick, 1999). Previous research has shown that periods of low fish recruitment coincide with turbid water conditions associated with the summer monsoon in Papua New Guinea (Srinivasan and Jones, 2006). If suspended sediment causes a reduction in the number of individuals available to settle, then coral reefs that are frequently exposed to high levels of suspended sediment may be more prone to recruitment failure.

However, there is the potential for numerous negative effects of increased suspended sediment. Too much turbidity can hinder visual cues, which is expected to affect an individual’s ability to forage (Fiksen et al., 2002; Wenger et al., 2012; Johansen and Jones, 2013). If high turbidity does ultimately reduce feeding efficiency in coral reef fish larvae, growth and body condition would be expected to decline with increasing sediment, while pelagic larval duration (PLD) should increase as development slows (e.g. Green and McCormick, 1999). Previous research has shown that periods of low fish recruitment coincide with turbid water conditions associated with the summer monsoon in Papua New Guinea (Srinivasan and Jones, 2006). If suspended sediment causes a reduction in the number of individuals available to settle, then coral reefs that are frequently exposed to high levels of suspended sediment may be more prone to recruitment failure.

Terrestrial run-off and associated increases in suspended sediment concentrations and turbidity are considered one of the major stressors to coral reefs (Burke et al., 2011), and it is crucial to understand the interplay between reduced water quality and coral reef fish development and recruitment patterns. In this study, we tested the hypothesis that increasing concentrations of suspended sediment would increase developmental time in the coral reef...
damselfish *Amphiprion percula* (Lacepède 1802) as a result of a reduction in foraging ability. To test this, we manipulated concentrations of suspended sediment under controlled aquarium conditions and measured effects on larval duration, size at settlement and mortality. The range of levels of suspended sediment employed in the experiment were set to reflect those recorded both in river plumes around coral reefs (Devlin et al., 2012) and on coral reefs during re-suspension events (Ogston et al., 2004; Browne et al., 2013).

RESULTS

Larval duration

The PLD was significantly longer in the sediment treatments compared with the control, with no significant differences among the sediment treatments (Kruskal–Wallis, $\chi^2=25.8$, $P<0.0001$; Fig. 1). The median age of metamorphosis among sediment treatments was 12 days, compared with 11 days in the control treatment. Although there was a relatively small difference in median age at metamorphosis between the sediment and control treatments, the frequency distribution curves for age at metamorphosis under increased sediment were consistently different from the control group (Kolmogorov–Smirnov test, $P<0.0001$). Almost 75% of the fish in the control had undergone metamorphosis by day 11, compared with 46%, 40% and 45% of the fish in the low, medium and high sediment treatments, respectively. By day 13, all of the fish in the control treatment had undergone metamorphosis compared with 63%, 80% and 70% of the fish in the low, medium and high sediment treatments, respectively. Two individuals in the high sediment treatment had a PLD of 22 days, twice the median PLD in the control.

Larval length and mass

Larvae in the low and high sediment treatments were significantly longer at metamorphosis than control fish (Kruskal–Wallis, $\chi^2=33.4$, $P<0.0001$; Fig. 2A). Larvae in the low and high sediment treatments were also significantly heavier at metamorphosis than those in the control treatment, and those in the low sediment treatment were significantly heavier than fish from the medium and high sediment treatments (Kruskal–Wallis, $\chi^2=36.1$, $P<0.0001$; Fig. 2B). There was a significant difference in length and mass among treatments at the chronological, as opposed to the developmental,
age (two-way ANOVA, $F_{4,256}=13.6, P<0.001$). For fish that settled on day 10, the fish in the low sediment treatment were significantly longer than the fish in the medium treatments, but were not significantly longer than the fish in the control and high sediment treatments. Additionally, the fish in the low sediment treatment were also significantly heavier than fish from all other treatments on days 10 and 11. The differences in sizes between the control and the sediment treatments at metamorphosis were driven in part by the continued growth of fish in the sediment treatments as they aged, resulting in fish that settled after or on day 14 being larger than fish that had settled earlier. Length at metamorphosis accounted for a significant amount of the variability in mass (ANCOVA, $F_{1,347}=41.1, P<0.0001$). When comparing mass for a standardized length among treatments, larvae in the low sediment treatment were significantly heavier than those in the control and high sediment treatments (ANOVA, $F_{3,347}=9.0, P<0.0001$; Fig. 2C). That is, at metamorphosis, regardless of age, the fish in the low sediment treatment had overall better body condition than the fish in the control and high sediment treatments. The range of mass of the fish in the medium sediment treatment overlapped with that of all treatments.

**Larval survivorship**

Mortality did not differ significantly among treatments (Kruskal–Wallis, $\chi^2=3.56, P=0.31$; Fig. 3). Survivorship was, on average, 34.4±3.32, 44.5±5.45, 32.6±5.45 and 42.6±4.64% (mean ± s.e.) in the control, low, medium and high sediment treatments, respectively.

**DISCUSSION**

The larval stages of marine fishes are likely to be susceptible to changes in water quality associated with increased sediment and nutrient loads. Much of the discussion in the literature has centered on the positive effects of increased nutrients driving increases in planktonic food (Lang et al., 1994; Grimes and Kingsford, 1996; Allman and Grimes, 1998). However, our study demonstrates that suspended sediment can cause a marked increase in the median and range in the PLD of a coral reef fish. Elevated sediment increased the median PLD by a day, and significantly increased the variation in PLD; in some cases PLD doubled from the normal 11 days to 22 days. Suspended sediment did not negatively affect length, mass, body condition at metamorphosis or survival to metamorphosis. Low levels of sediment led to increased size and enhanced body condition at metamorphosis, which may have been due to the increased ability to discriminate food particles. Sediment loading to coastal waters is likely to escalate with increasing coastal development (Tilman et al., 2001; Hamilton, 2010), creating higher levels of suspended sediment on coral reefs (Fabricius et al., 2013). This study provides evidence that suspended sediment levels currently reached in inshore areas could substantially change developmental patterns in a coral reef fish.

An increase in the PLD of marine organisms, even by a small amount, may have serious demographic consequences for a species because of the extremely high mortality rates of larvae (Houde, 1987; Bertram and Leggett, 1994). All else being equal, this would be expected to reduce the number of larvae surviving to age at metamorphosis and, consequently, lower recruitment success (Leggett and Deblois, 1994; Bergenius et al., 2002). Conversely, being larger and with better body condition may confer advantages during the first few days after metamorphosis and settlement when mortality can also be extreme (Perez-Dominguez and Munch, 2010). Longer PLDs could also lead to increased dispersal (Lester et al., 2007; Shanks, 2009). If so, increases in sediment may alter patterns of population connectivity on coastal reefs.

In our experiment, delayed metamorphosis and changes in growth and condition occurred at relatively low levels of suspended sediment. Exposure to such levels should be commonplace for communities on coastal reefs as peak fish recruitment overlaps with the wet season in both the Caribbean (Sponaugle and Cowen, 1996; Enfield and Alfaro, 1999) and the Great Barrier Reef (McCormick, 2003; Brodie et al., 2007). The effect of suspended sediment on coral reef fish larvae may be one of the driving mechanisms underlying distribution patterns seen across tropical continental shelves, from inner to outer reefs (Letourneau et al., 1998; Mallela et al., 2007). The increase in sediment loads is likely to affect both spatial and temporal patterns of coral reef fish distributions by reducing the number of coral reef fish larvae that can successfully settle on reefs experiencing elevated concentrations of suspended sediment (Wenger et al., 2011; Wenger and McCormick, 2013).

At this stage it is not known why larvae exposed to sediment have a longer development period. Therefore, what follows is a discussion on the potential mechanisms that could be driving the delay in reaching this developmental stage. Delays in metamorphosis have previously been described as a reduced growth rate once competency is reached, wherein the fish take the same amount of time to reach competency but then delay metamorphosis and continue to exist as larvae (Cowen, 1991; McCormick, 1999). Based on this definition of competency, it appears that the fish in the current study are delaying metamorphosis upon reaching competency. The fish in the sediment treatments were generally older, longer and heavier at metamorphosis than the fish in the
control treatment, indicating that they have surpassed a critical threshold for competency according to the above guidelines.

Prolonged larval development can be associated with poor environmental conditions, such as reduced food or sub-optimal temperatures, so that it takes longer to reach a state where the larvae are developmentally prepared for metamorphosis (e.g. McCormick and Molony, 1992; McCormick and Molony, 1995; Green and Fisher, 2004, McLeod et al., 2013). Sediment could have potentially affected larvae through an impairment of visual cues used for foraging, as was initially predicted as an outcome of this experiment. If increased sediment led to reduced foraging success then the results may be expected to more closely resemble the results of Green and McCormick (Green and McCormick, 1999) who showed that for A. melanopus, reduced food led to longer PLDs and smaller size at metamorphosis than in well-fed larvae. However, in this study, PLD was positively correlated with mass and body condition at metamorphosis. If body condition is considered a proxy for foraging success, then the current study has demonstrated that sediment increased foraging success for fish in the low sediment treatment. The delay in metamorphosis is therefore not associated with slower larval growth or poorer body condition as a consequence of suspended sediment. Interestingly, the fish in the high sediment treatment were also significantly longer and heavier than the control fish at metamorphosis, which contradicts previous work that showed suspended sediment impaired foraging in a coral reef fish at this level (Wenger et al., 2012). Although suspended sediment was not impairing foraging in this experiment, it was clearly affecting the larvae in other ways, resulting in extended PLDs. This result is particularly significant as it highlights an important knowledge gap. Although river plumes can promote higher growth of marine fish larvae as a result of nutrient enrichment (Lang et al., 1994; Allman and Grimes, 1998), no studies have examined the PLD of these larvae that derive benefit from river plumes. It may be that even though nutrient-enriched plumes provide more food, the effects of suspended sediment in the plume could still delay metamorphosis through mechanisms unrelated to foraging efficiency. More research is necessary to examine the relationship between river plumes and the settlement success of larvae that are entrained in them. Competency to settle can be dictated by a complex interaction of developmental parameters and environmental cues, unrelated to growth and body condition. It may be that suspended sediment is interfering with developmental processes important for metamorphosis, not related to age or size, such as sensory development (Shand, 1997; Lara, 1999), or that a decoupling of physiological features has occurred, such that some developed more slowly than is typical (Spicer and Burggren, 2003). There are different mechanisms that trigger metamorphosis and settlement in the wild, particularly environmental cues that indicate suitable habitat (McCormick, 1999; Pechenik, 2006). Although it is unknown what is triggering fish metamorphosis in the laboratory, it is evident that suspended sediment is changing the ability of the fish in the sediment treatments to metamorphose within the typical time frame of 11 days (Almany et al., 2007).

Another explanation for delayed metamorphosis may be that increased suspended sediment induces a response to delay metamorphosis and settlement until better environmental conditions are encountered or external cues necessary to trigger metamorphosis are detected. Previous studies have shown that suspended sediment impairs habitat choice at settlement in certain coral reef species through a reduction in visual cues (Wenger et al., 2011; Wenger and McCormick, 2013). Therefore, there is the potential that if coral reef fishes cannot successfully perceive visual cues necessary for habitat choice, they may delay metamorphosis. Marine invertebrates have been shown to delay metamorphosis in the absence of chemical or physical cues (reviewed in Pechenik, 1990). McCormick experimentally demonstrated that a coral reef fish close to settlement can delay metamorphosis if kept away from suitable habitat or settlement sites (McCormick, 1999). It may be that if the larvae are in sub-optimal conditions, such as reduced water quality, metamorphosis and settlement can be delayed. Delays in metamorphosis have previously been inferred through otolith analysis (e.g. Victor, 1986; Cowen, 1991). These studies have demonstrated, based on the variability in PLD and the size at settlement, that some species can be remarkably flexible in the timing of settlement. Plasticity in the timing of metamorphosis may be favored in environments that frequently experience suspended sediment, because of the variability in environmental conditions, particularly if individuals will be larger at settlement (Sponaugle and Pinkard, 2004; Perez-Dominguez and Munch, 2010); however, as discussed above, the consequences of delaying metamorphosis may be great. Importantly, there was no significant difference in the time to metamorphosis among fish from the low, medium and high sediment treatments, suggesting that the sediment threshold for behavioral changes may be close to conditions found relatively frequently in the natural environment (Table 1). More research is needed to determine the mechanism underpinning the delays in metamorphosis caused by suspended sediment and how a sediment-induced delay alters survivorship and recruitment patterns.

In conclusion, our study provides important evidence that suspended sediment can significantly alter a crucial life history stage of a coral reef fish. Even a relatively small increase in suspended sediment concentration prolonged larval development of A. percula and affected size at metamorphosis and body condition. Given the critical importance of this life history stage for recruitment and population viability, our study underscores the crucial role that suspended sediment may play in influencing reef fish population dynamics now and in the future.

<table>
<thead>
<tr>
<th>Location</th>
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<th>Reference</th>
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<td>120</td>
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<td>Van Woestik and Hopley, 1988</td>
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<tr>
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<td>5–45</td>
<td>Inshore reefs</td>
<td>Larcombe et al., 1995</td>
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<tr>
<td>GBR, Australia</td>
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<td>Flood plumes</td>
<td>Devlin et al., 2001</td>
</tr>
<tr>
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<td>0–100</td>
<td>Fringing reef</td>
<td>Ogston et al., 2004</td>
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<td>Coastal reefs</td>
<td>Wolanski et al., 2008</td>
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<td>GBR, Australia</td>
<td>0–300</td>
<td>Inshore reefs</td>
<td>Browne et al., 2013</td>
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GBR, Great Barrier Reef.
MATERIALS AND METHODS

Study species and brood stock maintenance

Five breeding pairs of *A. percula* (Pomacentridae) were captured from reefs from around the Cairns region of the northern Great Barrier Reef and transported to the Marine and Aquaculture Research Facilities Unit (MARFU) at James Cook University, Townsville, Australia. This species was used because they are easy to breed in captivity, have high survivorship as larvae and have relatively short pelagic larval durations. Breeding pairs were maintained in 60 l outdoor aquaria with a flow-through of filtered water (28.5°C) and fed twice per day with 0.075 g per pair of INVE Aquaculture Nutrition 12/20 pellets (Proaqua Pty Ltd, Queensland, Australia). Each breeding pair was provided with half a terracotta pot on the bottom of their aquarium for shelter and to serve as a structure for egg deposition. The pots were inspected each morning for the presence of eggs. This research was conducted under JCU ethics approval A1713.

Larval rearing conditions

The experiments were conducted between January and April 2012 at MARFU, which corresponds with the natural breeding time of this species. On the afternoon when hatching was predicted (6–8 days after eggs were laid), the pot containing the eggs was transferred to another 60 l aquarium inside a temperature-controlled (28.5°C) experimental lab, where hatching occurred within a few hours of darkness.

Larvae were reared in the 60 l aquarium (28.5°C) for 5 days in a semi-closed system; static during the day, then slowly flushed with filtered seawater each morning prior to light. Green *Nannochloropsis* spp. paste (Reed Mariculture, CA, USA) was added to the water each morning after flushing, until the bottom of the aquarium could not be seen, equating to ~4 million cells ml⁻¹ (Moorhead and Zeng, 2011). This was done to both dissipate light and maintain the nutritional value of the rotifers (*Brachionus* sp.) that were fed to the larvae at a density of 10 rotifers per ml each morning for the first 3 days. After the third day, the diet of the larvae was increasingly enriched with newly hatched *Artemia* sp. nauplii (INVE technologies, Thailand Ltd; GSL0) and the amount of rotifers they received decreased proportionally (G. Miller, personal communication).

Experimental apparatus and sediment suspension method

The sediment exposure trials were carried out in 24, 2 l polyethylene terephthalate (PET) bottles used as tanks. These readily available containers were chosen because of their food-safe characteristics and a shape that aided in the movement of suspended sediments. Each tank was fitted with a 1 mm plastic mesh screen that was used as a physical barrier to prevent larval loss from the tank, and shaded with black polyurethane sheeting to minimize light exposure. Each tank was also fitted with a partial plastic lid that reduced light glare and allowed for larval rearing without the addition of Green *Nannochloropsis* spp. paste (B. Green, personal communication). Each sediment treatment was delivered to individual tanks via a submersible pump (1200 l h⁻¹) placed in an external 100 l aerated sump. An airline was positioned directly in front of the suction inlet of this pump, which created consistent dissolved oxygen levels (~90%) to each larval rearing tank for the duration of each trial. Each sump contained three disturbance pumps (1000 l h⁻¹) to create uniform turbidity (Fig. 4A). Water inlet lines were arranged to allow for supply of clear (control) water as well as one of the three treatments. Water was delivered to each tank through low pressure, low flow inlet lines that were sufficient to maintain consistent sediment levels without disturbing the larvae (determined in a pilot study; A.S.W., unpublished data). Measurements using a WP88 Turbidity Meter (Thermo Fisher Scientific, QLD, Australia) during a pilot study showed that the constant supply of water with sediment in suspension from the inlet lines (185±19 s⁻¹) ensured that sediment concentrations remained constant in each tank throughout the experiment. A power-assisted drain line was incorporated to mitigate sediment deposition within the tanks and plumbing. A dual draining standpipe manifold was used to ameliorate sediment movement and to equilibrate water levels amongst all tanks. The design allowed for randomization of tanks between trials and treatments (Fig. 4B).

Tanks and sumps were arbitrarily assigned a sediment treatment between trials to eliminate any location bias within the experimental laboratory.

Experimental design and measurements of larval development

Larval development was measured at four different suspended sediment concentrations: control (0 mg l⁻¹; 0 nephelometric turbidity units, NTU), low (15 mg l⁻¹; ~2.5 NTU), medium (30 mg l⁻¹; ~5 NTU) and high (45 mg l⁻¹; ~7.5 NTU). A total of 20 replicates were run (1 replicate =1 tank) for each sediment treatment. The levels of suspended sediment for the four treatments were within the range of suspended sediment levels recorded in river plumes around coral reefs and on coral reefs during re-suspension events (Table 1). The higher sediment treatments were at levels that have been shown to affect the behavior and foraging ability of adult pomacentrid species (Wenger et al., 2012; Wenger and McCormick, 2013). The suspended sediment concentrations were maintained throughout the experiment, except for 1 h every 3 days when full water exchanges were conducted in order to remove waste. These water exchanges were completed before the first feeding of the day. The sediment used for the experiment was Australian bentonite, a commercially available clay. Muddy sediments and clays are common constituents of sediment found on inshore coral reefs, such as in the Great Barrier Reef (GBR) (Cartier et al., 1993; McCulloch et al., 2003) and the particle size of bentonite is in the same size class as particles found in suspension in the GBR (Bainbridge et al., 2012) (A.S.W., unpublished data).

On the fifth morning post-hatching (before feeding), larvae that were visually in good condition (i.e. displaying normal swimming behavior and balance) were gently collected using a glass beaker and arbitrarily distributed among the experimental tanks until there were 10 larvae in each tank. The decision to use 5 day old larvae in this study was based on a pilot study examining the interaction between the flow required to keep consistent levels of suspended sediment and the swimming ability of the larvae at different ages (A.S.W., unpublished data). During the pilot study, larvae were added at day 3 but could not cope with the flow in the tanks, resulting in 100% mortality within an hour. All larvae were fed once transfers were complete. Because the water was in constant motion and food was being removed from the tanks by the suction outlet, larvae from all treatments were fed the same food levels three times a day for the duration of the trial to ensure adequate feeding.
opportunity. Once all the larvae in the tanks displayed normal swimming behavior, sediment was slowly added to the treatment sumps.

Larvae were carefully checked for metamorphosis by torchlight each morning before they had an opportunity to feed. Larvae were considered as having undergone metamorphosis when their post-oralial stripe appeared, which coincided with a positive attraction to the sides of the rearing tanks consistent with settlement behavior and has been used as a diagnostic tool for metamorphosis and settlement in previous experiments using both A. percula (Dixson et al., 2008; McLeod et al., 2013) and a congeneric species A. melanopus (Green and McCormick, 1999). Once the larvae were considered settled they were removed from the tank and killed using an ice–saltwater slurry. These larvae were then immediately transferred to Bouin’s solution for 24 h and then transferred to 70% ethanol, at which point the following measurements were taken. Larvae were removed from the preservative, blotted dry, weighed (to the nearest 0.0001 g) and photographed in a lateral position on a 0.5 mm plastic grid. Standard length (SL) to the nearest 0.01 mm was estimated from each fish from the digital photograph using image analysis software (ImageJ v. 1.45s, National Institutes of Health, USA). Survival was measured as the difference between the number of larvae added to each tank at the beginning of each trial and the number of fish that remained at the end of each trial.

Data analysis
Analyses were performed based on both chronological age and age at metamorphosis. Chronological age refers to individuals that underwent metamorphosis on the same day. Age at metamorphosis refers to their pelagic larval duration, meaning the age the fish were when they metamorphosed. When the text refers to measurements made at metamorphosis, this means the age at metamorphosis. The distinction was made to assess whether length and mass were dependent on chronological age or on developmental stage, i.e. metamorphosis. A Kruskal–Wallis analysis with a Tukey’s HSD post hoc analysis was run to assess whether there were significant differences in: (i) median PLD, (ii) median standard length and mass at metamorphosis, and (iii) mortality among treatments. A two-way ANOVA was performed with treatment and age as factors to examine differences in mean standard length and mass at actual age. Analyses on differences at chronological age were only performed on fish aged day 10–13, as there were fish from each treatment that settled on these days. Two-sample Kolmogorov–Smirnov tests were run to compare the differences in the distribution of age of metamorphosis between the control and the sediment treatments. Body condition was calculated as the number of fish that remained at the end of each trial.

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References


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