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Facultative feeding in a marine copepod: effects of larval food and temperature on performance

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ABSTRACT: Most marine invertebrate larvae either feed or rely on reserves provisioned by parents to fuel development, but facultative feeders can do both. Food availability and temperature are key environmental drivers of larval performance, but the effects of larval experience on performance later in life are poorly understood in facultative feeders. In particular, the functional relevance of facultative feeding is unclear. One feature to be tested is whether starved larvae can survive to adulthood and reproduce. We evaluated effects of larval temperature and food abundance on performance in a marine harpacticoid copepod, *Tisbe* sp. In doing so, we report the first example of facultative feeding across the entire larval stage for a copepod. In a series of experiments, larvae were reared with ad libitum food or with no food, and at 2 different temperatures (20 vs. 24°C). We found that higher temperatures shortened development time, and larvae reared at higher temperature tended to be smaller. Larval food consistently improved early performance (survival, development rate and size) in larvae, while starvation consistently decreased survival, increased development time and decreased size at metamorphosis. Nonetheless, a small proportion (3–9.5%, or 30–42.7% with antibiotics) of larvae survived to metamorphosis, could recover from a foodless larval environment, reach maturity and successfully reproduce. We recommend that future studies of facultative feeding consider the impact of larval environments on adult performance and ability to reproduce.

KEY WORDS: Facultative feeding · Latent effects · Starvation · Larval nutrition strategies · Marine copepod · *Tisbe* sp.

1. INTRODUCTION

Development is initially fuelled by resources supplied by parents, but later costs are sometimes met by the offspring themselves; larval stages often feed in order to complete development. Marine organisms with complex life cycles exist somewhere on a continuum between 2 extremes, one in which feeding begins almost immediately, and the other in which larval feeding never occurs (Allen & Pernet 2007). Classic models describe these nutritional modes as a dichotomous trade-off between producing many small larvae from energy-poor eggs with a prolonged period of feeding in the plankton, and producing fewer, larger non-feeding larvae from energy-rich eggs (Vance 1973). Subsequent models have shown how the variation in maternal investment and intermediate nutritional strategies seen in nature may arise (e.g. McEdward 1997, Levitan 2000). Our focus here is on species with planktonic feeding larvae that can also develop without feeding — in other words, facultative feeders.

Facultative feeding is an intermediate between the extreme nutritional strategies of obligate feeding and non-feeding (Allen & Pernet 2007). Facultative feeding larvae are able to consume particulate food, and benefit from feeding, but do not require exogenous food to complete development to metamorphosis; these represent the 3 criteria for facultative feeding as proposed by Allen & Pernet (2007). Facultative

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feeding is considered rare in marine invertebrates, and has been confirmed based on these criteria in only 8 species (3 echinoderms, 4 molluscs and 1 annelid). Allen & Pernet (2007) listed the larvae of 2 decapods, another echinoderm, and 11 molluscs as potential new cases, but it is unclear whether they fulfil all 3 criteria. Since their review, an additional echinoderm and annelids of the genus *Capitella* have also been reported as potential facultative feeders (Table 1). Given the hundreds of species for which developmental data are available (Marshall et al. 2012), facultative feeding is almost certainly relatively rare.

Facultative feeding also need not occur across the entire larval phase. Larvae may depend on stored resources — either from yolk or acquired during earlier larval stages — to develop through some but not all larval stages, while retaining the ability to ingest food. 'Facultative lecithotrophy' has been used to refer to facultative feeding larvae in the crustacean literature, although sometimes the term is used to describe facultative feeding within individual larval stages rather than across the entire larval phase. We also include these examples of what we call 'partial facultative feeding' in Table 1. Studies of facultative lecithotrophy in decapods suggest a reduced reliance on planktonic food in these taxa, and may be associated with adaptation to non-marine environments and deep-sea and polar habitats (Allen & Pernet 2007).

The consequences of facultative feeding vary between taxa. Relative to obligate feeders, facultative feeders can have higher survivorship (in the absence of food), an extended period of larval competence,

Table 1. Reported cases of facultative feeding expanding the collation by Allen & Pernet (2007), and of partial facultative feeding restricted to certain larval stages in decapods. Partial facultative feeding is either primary facultative lecithotrophy (reliance on yolk reserves), or secondary (reliance on reserves from earlier larval stages) where specified. New cases not reported by Allen & Pernet (2007) are identified with an asterisk (*)

Taxa	New since Allen & Pernet (2007)	Facultative feeding for whole or part of larval phase	Reproduction observed in starved individuals	References
Annelida				
Polychaeta				
Capitella spp.	*	Whole	No	Adkins & Schulze (2011)
Streblospio benedicti		Whole	No	Pernet & McArthur (2006)
Arthropoda				
Decapoda				
Alpheus heterochaelis[a]		Whole	No	Knowlton (1973)
Armases angustipes	*	Partial	No	Anger (2001)
Armases miersii	*	Partial	No	Anger (1995a), Schuh & Diesel (1995)
Armases roberti	*	Partial	No	Anger (2001), Diesel & Schuh (1998)
Callianassa tyrrhena[a]		Whole	No	Thessalou-Legaki et al. (1999)
Exopalaemon carinicauda	*	Partial	No	Zhang et al. (2015)
Lepidophthalmus louisianensis	*	Partial	No	Nates & McKenney (2000)
Lepidophthalmus siriboia	*	Partial (secondary)	No	Abrunhosa et al. (2008)
Lysmata amboinensis	*	Partial	No	Cunha et al. (2008) but see Calado et al. (2007a)
Lysmada boggessi	*	Partial	No	Calado et al. (2007a)
Lysmata seticaudata	*	Partial (primary and secondary)	No	Calado et al. (2007a), Calado et al. (2007b)
Lysmata vittata	*	Partial	No	Almeida (2017)
Mithraculus forceps	*	Partial (secondary)	No	Figueiredo et al. (2008)
Palaemon elegans	*	Partial (secondary)	No	Calado et al. (2010)
Palaemon serratus	*	Partial (secondary)	No	Calado et al. (2010)
Palaemonetes argentines	*	Partial	No	Ituarte et al. (2005)
Palaemonetes pugio	*	Partial	No	Broad (1957)
Palaemonetes varians	*	Partial (primary and secondary)	No	Calado et al. (2010), Oliphant et al. (2013), Oliphant & Thatje (2013)
Pleuroncodes monodon	*	Partial	No	Espinoza et al. (2016)
Sesarma curacaoense	*	Partial	No	Anger (1995b)
Sesarma rectum	*	Partial	No	Anger (2001)
Sesarma reticularum	*	Partial	No	Staton & Sulkin (1991)

(Table continued on next page)

Table 1 (continued)

Taxa	New since Allen & Pernet (2007)	Facultative feeding for whole or part of larval phase	Reproduction observed in starved individuals	References
Echinodermata				
Asteroidea				
Henrica abyssicola	*	Whole	No	Benítez-Villalobos et al. (2007)
Echinoidea				
Brisaster fragilis[a]		Whole	No	Strathmann (1979), Hart (1996)
Brisaster latifrons		Whole	No	Hart (1996)
Clypeaster rosaceus		Whole	No	Emlet (1986), Allen et al. (2006)
Leodia sexiesperforata		Whole	No	Heyland et al. (2004)
Encope michelini		Whole	No	Eckert (1995)
Ophiuroidea				
Macrophiothrix rhabdota		Whole	No	Allen & Podolsky (2007)
Mollusca				
Bivalvia				
Codakia orbicularis[a]		Whole	No	Gros et al. (1997)
Pandora inaequivalis[a]		Whole	No	Allen (1961)
Gastropoda				
Adalaria proxima		Whole	No	Kempf & Todd (1989)
Aeolidia papillosa[a]		Whole	No	Williams (1980)
Alderia willowi		Whole	No	Botello & Krug (2006)
Berthelinia caribbea[a]		Whole	No	Grahame (1969)
Conus pennaceus		Whole	No	Perron (1981)
Dendronotus frondosus[a]		Whole	No	Sisson (2005)
Fasciolaria audouini[a]		Whole	No	Gohar & Eisawy (1967)
Hermissenda crassicornis[a]		Whole	No	Williams (1980)
Murex ramosus[a]		Whole	No	Gohar & Eisawy (1967)
Murex incarnates[a]		Whole	No	Gohar & Eisawy (1967)
Phestilla sibogae		Whole	Yes	Kempf & Hadfield (1985), Miller (1993)
Spurilla neapolitana[a]		Whole	No	Clark & Goetzfried (1978)
Tenellia pallida[a]		Whole	No	Eyster (1979)

[a]Potential, but not confirmed, facultative feeders as identified by Allen & Pernet (2007)

increased egg size, decreased development time and smaller clutch size as adults (Emlet 1986, Allen & Pernet 2007). There is considerable evidence that nutritional stress during larval stages reduces post-metamorphic performance in a range of taxa (e.g. Pechenik et al. 2002, Phillips 2002, 2004, Thiyagarajan et al. 2003, Emlet & Sadro 2006, Chiu et al. 2007, 2008). Latent effects, i.e. differences in performance in juvenile and adult stages driven by conditions during the larval phase (Pechenik 2006, Pechenik 2018), are less-well studied in facultative feeders. In a rare example, Calado (2008) showed how despite larger size at settlement, larvae starved during a facultative feeding phase exhibited poorer growth as juveniles relative to those that were fed as larvae. We therefore expect the complete absence of larval food to impact adult performance in facultative feeders.

The consequences of completing larval development without food for later life stages of facultative feeders are poorly understood. Out of the 46 reports and potential cases of facultative feeding, the ability to reproduce after metamorphosing without exogenous food has only been demonstrated in a single species (Table 1). We agree with Allen & Pernet (2007) regarding the list of criteria for demonstrating facultative feeding, but for facultative feeding to be functionally relevant, facultative feeding larvae also need to be able to reach maturity and successfully reproduce after metamorphosing without exogenous resources. Given that larval experiences can affect post-metamorphic performance more generally (Pechenik 2006, 2018), facultative feeders that survive starvation during the larval phase may be demographically irrelevant if they never survive to reproduce. By rearing starved larvae of *Phestilla sibogae* to adulthood and observing successful reproduction, Miller (1993) was able to rule out this possibility, but whether these effects are universal is unclear.

The costs and benefits of larval feeding are strongly affected by temperature. In marine invertebrates, temperature drives major life history patterns: offspring are more likely to be feeding larvae, and to be smaller, in warmer temperatures relative to cold (Marshall et al. 2012). Recent work has also shown how the net energy cost of development may arise from the temperature-dependence of metabolic rate and development time, such that costs of development are generally higher at cooler temperatures (Pettersen et al. 2019). Temperature may therefore be an important mediator of the costs of developing in the absence of food for a facultative feeder. However, few studies have examined the interaction between temperature and food in facultative feeders.

Here we document the first incidence of facultative feeding in a copepod. Copepods are a diverse, ubiquitous group of crustaceans that play a critical role in marine food chains and the ocean carbon pump (Turner 2015), and are well suited to laboratory study. Copepod larval development is varied, exhibiting both non-feeding and feeding strategies (Strathmann 1985), but whether these strategies are obligate or facultative has not previously been evaluated to our knowledge. The aim of this study was to explore the pre- and post-metamorphic consequences of food-replete and food-absent environments, including whether development in the absence of larval food affects the capacity to reproduce as adults. Finally, we explored how different temperature modifies the effect of larval food on development in a facultative feeder.

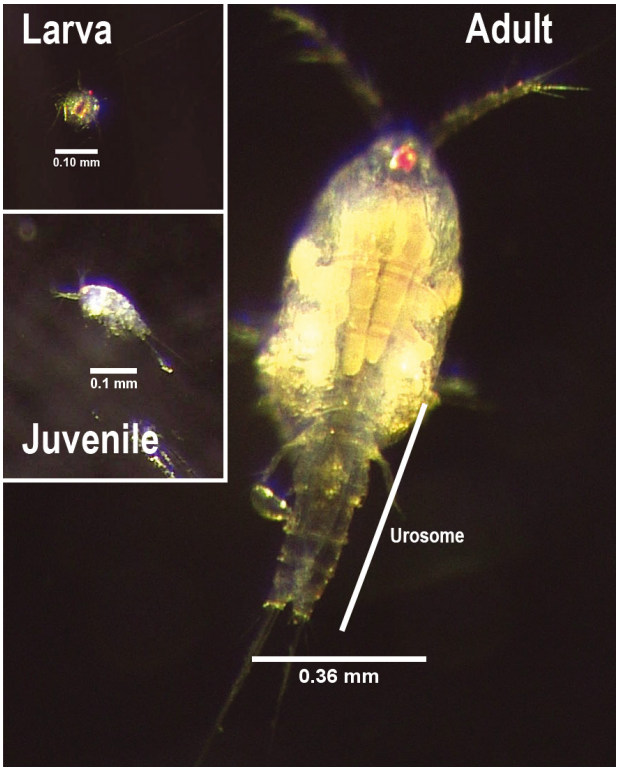

Fig. 1. Key life stages of *Tisbe* sp. Naupliar stage 1 (NI) larvae were collected and allocated to treatments until reaching the first post-larval juvenile stage (CI). CI copepodites (juveniles) were counted and measured, and reared until adulthood (CVI). The number of segments in the urosome is indicative of copepodite stage

2. MATERIALS AND METHODS

2.1. Study organism

Tisbe sp. is a littoral marine copepod from the family Tisbidae (Arthropoda: Harpacticoida), and has not been resolved to species level in the Southern Ocean (D. McKinnon pers. comm.). Our cultures have been maintained under laboratory conditions since founding populations were collected in 2017 from Brighton marina, Melbourne, Australia. The cultures are oxygenated, reared on the marine microalga *Dunaliella tertiolecta* and cleaned of detritus twice a week. Like other harpacticoids, *Tisbe* sp. develops through 6 larval naupliar stages (NI to NVI) and 6 post-larval copepodite stages (CI to CVI), with CVI being the adult (see Fig. 1 for key life stages; Dahms & Qian 2004). We refer to the larval stages as larvae, the post-metamorphic immature stages as juveniles and the mature stage as the adult. NI is generally non-feeding in harpacticoids, while feeding structures present from NII onwards suggest planktotrophy (Dahms 1990). Whether planktotrophy in the NII to NVI stages is obligate or facultative is unknown.

2.2. Experimental design

2.2.1. Overview of experiments

We used different protocols across 3 separate experiments (Table 2), as we were interested in exploring a range of different factors associated with facultative planktotrophy. Expt 1 examined whether larvae could complete development in the absence of food. Expt 2 examined the interaction between temperature and larval starvation on larval performance and metamorphosis, and was repeated in 2 separate runs. Expt 3 examined the effects of larval temperature, larval starvation and juvenile food levels on adult performance. After each experiment, we continued

Table 2. Summary of experimental protocols. Expt 1 focussed on the effects of starvation on larval performance, and Expt 2 additionally considered the effects of temperature on larval performance and was repeated once. In both Expts 1 and 2, individuals were reared with ad libitum food post-metamorphosis until reproduction or death occurred. Expt 3 considered how the larval and juvenile environments affected additional measures of adult performance — adult size, survivorship, time to maturity, egg sac size, egg size and first clutch size. Larvae were allocated to vials receiving food or no food, which were placed in waterbaths in Expts 2 and 3. Vials per food level are per waterbath for Expts 2 and 3

Experiment	Run	Waterbaths per temperature	Vials per food level	Larvae per vial	Temperature treatment	Antibiotics	Reproduction observed in all treatments	Multiple measures of adult performance
1	–	–	3	250	No	No	Yes	No
2	1	3	1 fed, 7 starved	15	Yes	Yes	Yes	No
2	2	3	1 fed, 3 starved[a]	50	Yes	Yes	Yes	No
3	–	3	1	49	Yes	No	Yes	Yes

[a]One starvation treatment vial was lost in run 2 of Expt 2

to rear starved larvae to adulthood to determine whether they could successfully reproduce.

We sought to create larval cultures that were free of potential food sources. For each experiment, approximately 200–250 fed gravid females were collected from culture jars. Mothers were individually pipetted into a petri dish filled with approximately 10 ml of pasteurised, 0.22 µm filtered seawater (FSW), and then individually pipetted again into a second petri dish to minimise the amount of detritus transferred. They were then washed in FSW and retained on a 100 µm mesh. Mothers were then rinsed into a beaker of 50–100 ml FSW, and were not given food. Every 24 h, these mothers were filtered again to remove and collect hatched larvae. This ensured that when a cohort of larvae was collected, no individuals within the cohort were over 24 h old. Consequently, larvae were starved for no more than 24 h before random allocation to experimental treatments. Larvae were counted and transferred by pipetting in small aliquots (10–20 µl), of 5 or 10 individuals, to minimise uptake of detritus into glass vials. These vials had been acid-washed with 10% HCl, scrubbed and rinsed with reverse-osmosis water to eliminate any potential food sources, then filled with 10 ml of FSW. The initial number of larvae per vial varied between experiments due to logistical constraints (e.g. number of spawning females) but were constant within any one experiment.

Fed treatments received approximately 5×10^6 *D. tertiolecta* cells in total (initial food concentration of 500 000 cells ml^{-1}), and all vials were covered with Parafilm between daily observations to minimise evaporation. Vials were checked daily under a dissecting microscope to count and remove all juveniles (assessed visually), and to remove moults and dead individuals until all larvae had metamorphosed or died. Water was changed only in vials still containing nauplii after 1 wk.

2.2.2. Expt 1 – Larval development in the presence and absence of food

In the first experiment, we simply wished to compare the effects of food-replete and -absent environments on larval development time and survival to metamorphosis, and determine whether starved individuals could successfully mature and reproduce. Six vials, each containing 250 larvae, were randomly allocated to fed or starved treatments (3 vials per food treatment). Survival in each vial was calculated as the number of individuals successfully reaching the first juvenile stage divided by the initial number of nauplii per vial. Larval development time was calculated as the mean time from commencing treatments to metamorphosis. Surviving juveniles were then separated from remaining nauplii and reared within the same cohorts in sterile plastic culture trays containing 4 ml of FSW to determine whether starved larvae could reach maturity and reproduce at all. Water was changed weekly and food was provided ad libitum (approximately 2500 *D. tertiolecta* cells ml^{-1} $ind.^{-1}$ wk^{-1}). Trays were checked every 3 d until reproduction had occurred at least once in each tray (replicate) or all individuals had died, after which the experiment ended.

2.2.3. Expt 2 – Effects of larval food and temperature on performance

In Expt 2, we were also interested in how temperature interacts with food availability for larvae, as re-

cent work has suggested that slight increases in temperature may reduce the cost of larval development (Pettersen et al. 2019). To evaluate the effects of different larval environments, we allocated vials of starved and fed larvae to waterbaths maintained at high (24°C) or low (20°C) temperature, using 3 waterbaths per temperature. We then allocated 1 vial of fed larvae and 7 vials of starved larvae to each waterbath in the first run, and in the second run we allocated 1 vial of fed larvae and 3 vials of starved larvae per waterbath. To ensure temperatures were stable, waterbaths were maintained for at least 3 d prior to allocation of larvae to experimental treatments, and temperatures were monitored each day using thermometers.

After the first experiment, it became clear that larvae could complete development without phytoplankton. While we suspected that bacterial populations were low in our treatments, we wanted to rule out the possibility that bactivory was allowing our larvae to complete development. Thus, in Expt 2, we added 40 µl of 10 000 units ml^{-1} (approximately 6 mg ml^{-1}) penicillin G and 10 mg ml^{-1} streptomycin solution (Sigma-Aldrich) to each vial of larvae to inhibit bacterial growth that could provide an unaccounted source of larval food (Rieper 1978).

We measured larval development time and survivorship to metamorphosis in the same way as in Expt 1. Additionally, we measured post-metamorphic size by collecting individuals within 24 h of metamorphosing and photographing them using a Motic Moticam 1080 camera mounted on an Olympus SZ61 dissecting microscope. Stage 1 juvenile body length was measured using FIJI (Rueden et al. 2017). Juveniles were reared to adulthood as per Expt 1, until either death or reproduction was observed in each treatment (but not necessarily in each replicate), after which the experiment ended.

2.2.4. Expt 3 – Effects of larval and juvenile food and temperature on adult performance

To evaluate the effects of larval and juvenile environments on adult performance, larvae were allocated to food-replete or food-absent vials in high (24°C) or low (20°C) temperature waterbaths as per Expt 2. One vial per food level, each vial containing 49 larvae, was allocated to each waterbath (3 waterbaths per temperature, hence 3 replicates). Larval development time and survivorship were recorded as per Expt 1. Antibiotics were not used in Expt 3.

Juveniles were removed within 24 h of metamorphosing. These juveniles were transferred to sterile plastic cell culture trays containing 4 ml of FSW and remained within their cohorts, i.e. all mates came from the same vial. This was done to maximise the probability of obtaining at least 1 mating pair from each replicate, but consequently juvenile density was not controlled for and was generally lower in cohorts from larval starvation treatments. We assumed that the effects of crowding would be minimal due to large volume per capita (relative to stock populations), and food was provided on a per capita basis. Juveniles were subjected to either ad libitum feeding or food-limited treatments (approximately 2500 or 1250 *D. tertiolecta* cells ml^{-1} $ind.^{-1}$ wk^{-1}, respectively) and fed daily. Salinity was controlled by measuring evaporative weight loss in blank trays once a week, and then adding the weight difference in reverse osmosis water to each tray. Every 2 wk, individuals were pipetted into fresh trays and water to minimise growth of potential pathogens. Trays were checked daily for fresh moults and dead individuals, which were counted and removed. Abdominal segments of intact moults were counted under a dissecting microscope to determine whether the adult stage had been reached.

Time to reproductive maturity within each tray was measured as days from metamorphosis to when females were first gravid or when a moult was collected with 5 segments (somites) in the urosome (Fig. 1). Survival was censused on first observation of egg production in each tray. As such, not all individuals in each replicate were necessarily adults. However, because we were unable to visually identify maturity (or sex) in non-gravid live copepods, we considered this to be a reasonable approximation for time to maturity for a same-aged cohort. Survival from metamorphosis was measured by dividing the number of living individuals at census time by the initial number of individuals allocated to that tray, and body length was measured for all individuals. For gravid females, fecundity was measured as the dorsoventral egg-sac area, and egg size was the mean diameter of 5 eggs. These females were reared individually without changing their per capita food provisioning until their offspring hatched and the size of their first clutch was measured.

2.3. Statistical analysis

Data were evaluated graphically to ensure statistical assumptions were met as per Quinn & Keough (2002). Homoscedasticity was evaluated using QQ plots and fits vs. residuals. For Expt 1, type III ANOVAs were used to evaluate food treatment as a

fixed effect on larval survival and development time. Expt 2 was analysed using type III ANOVAs to evaluate differences in larval survival, time to metamorphosis and size immediately after metamorphosis. The interaction of food and temperature treatments was analysed as fixed effects, with waterbath nested within temperature, and run was treated as a fixed block effect. Larval survival and development time were analysed in the same way for Expt 3, but no run effect was necessary. For adult performance, due to high larval mortality in the starvation treatment, we had insufficient surviving replicates to test the complete model for our partially nested experimental design (Quinn & Keough 2002). Instead, we tested adult performance in Expt 3 using larval and juvenile food levels and their interaction as fixed factors, with the effect of replicate nested in temperature as a fixed block effect. Analyses for Expt 1 and adult performance in Expt 3 were performed using R version 3.6.1 (R Core Team 2018) and the 'Car' package version 3.0-2 (Fox & Weisberg 2011). Analyses for Expt 2 and larval performance in Expt 3 (Appendix 1) were done using Minitab version 19.2 (Minitab 2019), fitting an ANOVA model for a split-plot design. In addition to statistically significant ($p < 0.05$) results, we highlight marginally non-significant results where p was between 0.05 and 0.10.

3. RESULTS

3.1. Expt 1 – Larval development in the presence and absence of food

Fed larvae performed better than starved larvae (Table 3). Copepod larvae that were fed survived better (Fig. 2B) and developed approximately 2 times faster (Fig. 2A) than unfed larvae. Nonetheless, 4.3% of unfed larvae reached metamorphosis (compared to 92.8% of fed larvae), and with subsequent addition of ad libitum food, also reached adulthood and successfully reproduced. Reproduction was observed at least once per tray (replicate).

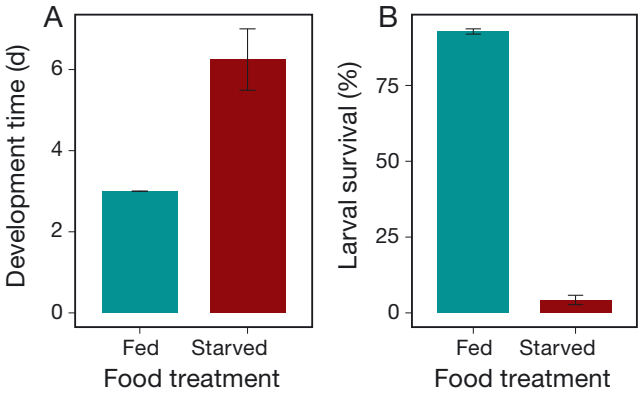

Fig. 2. Effect of starvation on larval performance (Expt 1). Larval performance was measured in terms of (A) development time and (B) survival to post-larval stage. Bars represent means of 3 replicates under starved/fed treatments. Performance was consistently higher in fed treatments, but ~4.3% of larvae survived starvation and metamorphosed, suggesting facultative planktotrophy. Error bars show between-vial standard deviation

3.2. Expt 2 – Effects of larval food and temperature on performance

Larvae that were fed and larvae reared at higher temperature performed better (Table 4). Consistent with Expt 1, time to metamorphosis was shorter (Fig. 3A) and survivorship was higher (Fig. 3B) when larvae were fed and reared at higher temperature. Nonetheless, 30.0–42.7% of starved larvae successfully metamorphosed (compared to 90.7–95.6% of fed larvae). Larvae that were fed were also larger as juveniles. Although the effect was marginally non-significant, juvenile size also tended to be smaller in individuals reared at a higher larval temperature (Fig. 3C). There were no significant interaction effects, and there was a significant effect of run on development time. Successful reproduction was observed at least once in each treatment (no replication).

Table 3. Type III SS ANOVAs for larval performance in Expt 1. Survivorship was from hatching to the first juvenile stage, and development time was from hatching to metamorphosis. An asterisk (*) indicates statistically significant p-values

Response	Effect	df	Mean squares	*F*	p
Survivorship	Food	1	11757.2	5010.2	<0.001*
	Residuals	4	2.35		
Development time	Food	1	11.2	26.01	<0.01*
	Residuals	4	0.425		

3.3. Expt 3 – Effects of larval and juvenile food and temperature on adult performance

Adult performance was higher in individuals that had been provisioned ad libitum food rather than a restricted

Table 4. Type III SS ANOVAs for larval performance in Expt 2. Survivorship was from hatching to the first juvenile stage, and development time was from hatching to metamorphosis. First juvenile stage size was moult length. An asterisk (*) indicates statistically significant p-values

Response	Effect	df	Mean squares	*F*	p
Survivorship	Run	1	3.0	0.06	0.82
	Temperature	1	36.6	0.68	0.43
	Whole plot error	9	51.8	0.48	0.86
	Food	1	17097.7	156.97	<0.001*
	Food × Temperature	1	163.8	1.50	0.25
	Subplot error	9	108.9		
	Total	22			
Development time	Run	1	0.9735	9.22	<0.05*
	Temperature	1	0.9819	9.30	<0.05*
	Whole plot error	9	0.1056	0.59	0.78
	Food	1	42.7020	238.71	<0.001*
	Food × Temperature	1	0.3783	2.11	0.18
	Subplot error	10	0.1789		
	Total	23			
First juvenile moult size	Run	1	0.000160	1.01	0.34
	Temperature	1	0.000575	3.65	0.09
	Whole plot error	9	0.000158	0.68	0.71
	Food	1	0.026931	116.53	<0.001*
	Food × Temperature	1	0.000136	0.59	0.46
	Subplot error	10	0.000231		
	Total	23			

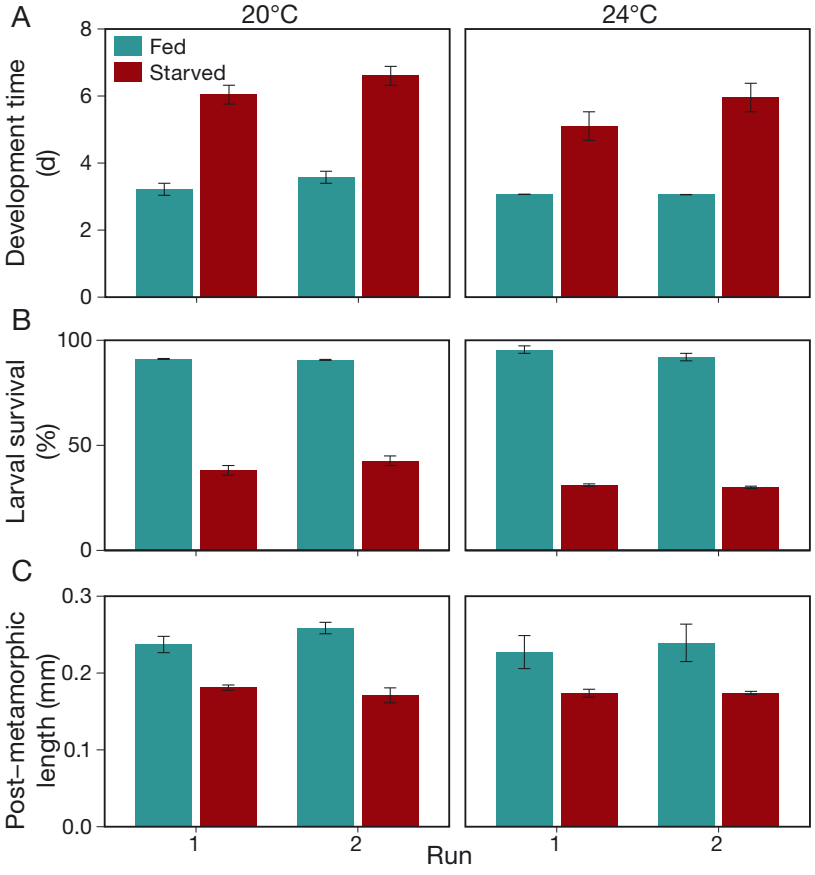

Fig. 3. Effects of starvation and temperature on larval performance (Expt 2). Larval performance was measured in terms of (A) development time, (B) survival to post-larval stage and (C) post-larval performance as first juvenile moult length, immediately after metamorphosis. Bars represent means of 3 replicates under starved/fed and high/low temperature treatments. Performance was generally higher in fed and warmer treatments, but juvenile size tended to be smaller at warmer larval rearing temperature. Of starved larvae, ~30.0–42.7% successfully metamorphosed without food, suggesting facultative planktotrophy. Error bars show between-run standard deviation

quantity of food as juveniles (Fig. 4, Table 5), with some caveats. However, we found no effects of larval food level on several adult traits, including adult size, egg sac size and egg size. Statistically significant (or marginally non-significant) effects of larval or juvenile food provisioning were observed in time from metamorphosis to maturity, survival from metamorphosis to maturity, and first clutch size. The only significant interaction effect was in first clutch size. Larval results for Expt 3 are presented in Appendix 1.

Time from metamorphosis to maturity was shorter for individuals fed ad libitum as juveniles, regardless of their larval food regime Fig. 4A). While marginally non-significant, mortality also tended to be lower for individuals fed ad libitum as juveniles, and lower in individuals that had been starved as larvae (Fig. 4B).

There was an interaction between larval and juvenile food regimes on first clutch size (Fig. 4C) as well as a significant effect of waterbath (nested within temperature) (Fig. 4D). First clutch size was generally higher in mothers that had received food as larvae, but mothers that had been starved as larvae produced smaller clutches when fed ad libitum as juveniles compared to those with restricted juvenile food intake.

Additionally, differences in egg size between individuals starved as larvae

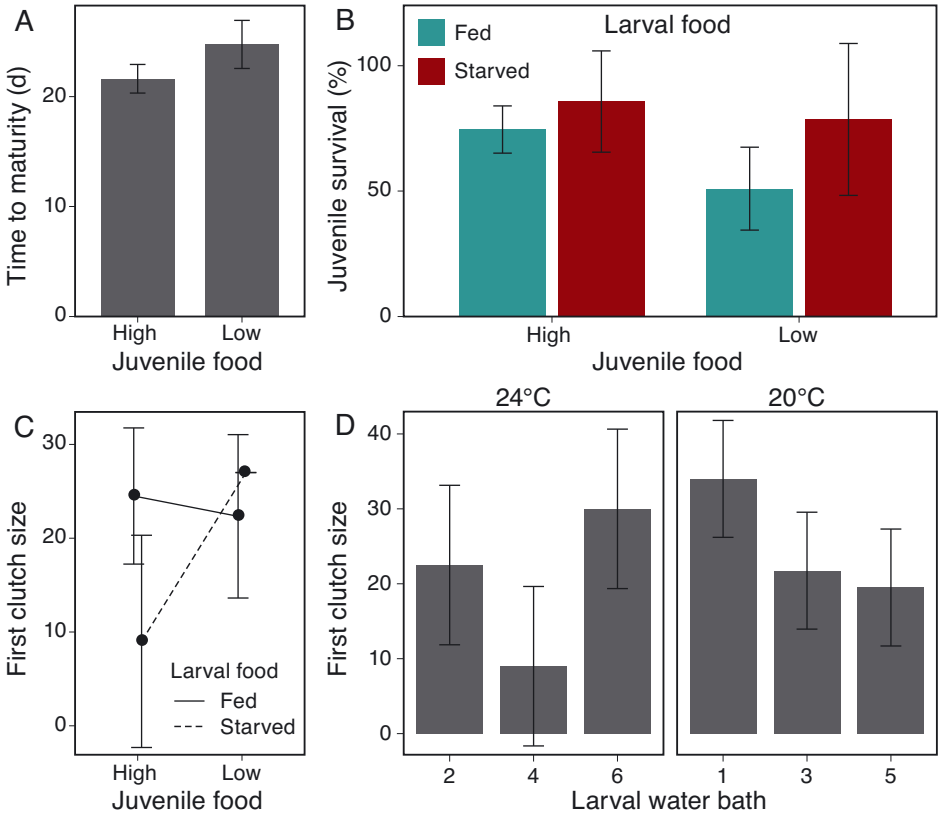

Fig. 4. Effects of larval and juvenile food provisioning, and larval temperature, on adult performance (Expt 3). Adult performance was measured in terms of (A) time from metamorphosis to maturity, (B) survival from metamorphosis to maturity, (C) size of first clutch showing an interaction between larval and juvenile food treatments and (D) the effect of waterbath in larval temperature treatments. Bars and dots represent means of replicates within waterbaths, which varied in number due to high mortality in unfed larvae. The effects of larval food and temperature treatments are presented separately, as the data were too sparse for both effects to be included in a single statistical model. N = 8 for each treatment in panels A and B; N = 3 for fed larval treatments, N = 2 at high juvenile food and 1 at low juvenile food in starved larval treatments in panel C; and N = 2 replicates for waterbaths 1, 2, 5, and 6 (all starved larvae died), 4 replicates for waterbath 3 and 3 replicates for waterbath 4 in panel D. Error bars indicate standard deviation

may have differed according to larval rearing temperature and juvenile food provisioning. However, these differences were not statistically tested, as the data were too sparse for formal analysis, due to high mortality in the food-absent larval treatments (Appendix 2).

4. DISCUSSION

Larvae reared in food-replete environments and in warmer temperatures performed better. With feeding, larvae of *Tisbe* sp. experienced higher survival, shorter development times, and larger post-larval size compared to those subjected to starvation, and larvae reared at higher temperature developed faster. Broadly, these findings are consistent with other studies of the effects of food provisioning and temperature on copepod larvae (Paffenhöfer 1970, Williams & Jones 1994, Campbell et al. 2001, Cook et al. 2007). A small proportion of larvae reared in food-absent environments survived to metamorphosis, successfully reached maturity and reproduced. This is the first reported evidence for facultative feeding in the larvae of a copepod, to our knowledge, and the second reported case of facultative feeding larvae metamorphosing without exogeneous food and successfully reproducing. While low larval food concentrations generally impact juvenile performance in crustaceans and other marine invertebrates (Pechenik 2006, 2018), we found little evidence for latent effects of larval environments on adults; but given the very low numbers of survivors (2–8) in some treatments, we do not attempt to draw statistical inference from these results in particular. Nonetheless, adults reared in food-replete and -absent larval environments tended to show similar performance, suggesting that juveniles starved as larvae may exhibit compensatory growth to catch up to individuals from favourable larval environments (Metcalfe & Monaghan 2001).

4.1. Larval mortality

Larvae survived in the absence of food, but their survival was markedly lower than for larvae that were fed. Only a small proportion (4–15%) of nauplii died in our high-food treatments, similar to survival in other copepod larvae reared under ad libitum feeding conditions (Jamieson 1986, Almeda et al. 2010, Koch et al. 2017). Facultative feeders generally exhibited high survivorship to metamorphosis when

Table 5. Type III SS ANOVAs for adult performance in Expt 3. Time to maturity and survivorship is from metamorphosis to adulthood, and size at maturity is body length. In reproductive mothers, fecundity is the size of the first egg sac, and egg size is the mean diameter of 5 randomly sampled eggs from a photograph of the egg sac. Waterbath is nested within temperature. An asterisk (*) indicates statistically significant p-values

Response	Effect	df	Mean squares	*F*	p
Time to maturity	Juvenile food	1	35	13.22	<0.01*
	Larval food	1	6.1	2.31	0.17
	Juvenile food×Larval food	1	1	0.39	0.55
	Waterbath(Temperature)	5	4.46	1.68	0.26
	Residuals	7	2.642857		
Survivorship	Juvenile food	1	708.2	3.71	0.096
	Larval food	1	974.5	5.10	0.058
	Juvenile food×Larval food	1	202.8	1.06	0.33
	Waterbath(Temperature)	5	360.28	1.89	0.21
	Residuals	7	191.0429		
Size at maturity	Juvenile food	1	0.0009	0.52	0.49
	Larval food	1	0.000005	0.003	0.96
	Juvenile food×Larval food	1	0.00008	0.05	0.84
	Waterbath(Temperature)	5	0.0008	0.50	0.77
	Residuals	7	0.001429		
Fecundity	Juvenile food	1	0.00009	0.48	0.51
	Larval food	1	0.0005	2.53	0.16
	Juvenile food×Larval food	1	0.000001	0.006	0.94
	Waterbath(Temperature)	5	0.00008	0.45	0.80
	Residuals	6	0.000167		
Egg size	Juvenile food	1	0.0002	3.02	0.13
	Larval food	1	0.000008	0.11	0.75
	Juvenile food×Larval food	1	0.00003	0.35	0.57
	Waterbath(Temperature)	5	0.00006	0.69	0.65
	Residuals	6	8.33×10^{-5}		
First clutch size	Juvenile food	1	53.4	3.40	0.11
	Larval food	1	6	0.38	0.56
	Juvenile food×Larval food	1	107.6	6.85	<0.05*
	Waterbath(Temperature)	5	135.34	8.62	<0.05*
	Residuals	6	15.7		

starved through the larval phase compared to when fed. Previous studies have found that for starved and fed larvae, respectively, survival rates to metamorphosis were 83.9 and 82.7% for *Conus pennaceus* (Perron 1981); 97.6% and 86.9 for *Clypeaster rosaceus* (Emlet 1986); 20 and 34% for *Adalaria proxima* (Kempf & Todd 1989); 93 and 94% for *Phestilla sibogae* (Miller 1993); 76 and 68% for *Callianassa tyrrhena*, (Thessalou-Legaki et al. 1999); and 45.3 and 80.4–100%, depending on food type, for *Macrophiothrix rhabdota* (Allen & Podolsky 2007). Some facultative feeding larvae exhibit markedly lower survivorship in the absence of exogeneous food. Eckert (1995) found that the feeding larvae of the sand dollar *Encope michelini* can consistently develop all larval structures to the final (8-armed) larval stage independent of food availability, and that a small proportion (1.7%) could metamorphose without an external source of food. Similarly, Heyland et al. (2004) found low rates of successful metamorphosis in the absence of food in another sand dollar with feeding larvae, *Leodia sexiesperforata*. However, this ability to metamorphose without food was observed only in offspring derived from sufficiently large eggs (observed in 12% of offspring from larger eggs). Survival rates in *E. michelini* and *L. sexiesperforata* are comparable to those we found in unfed *Tisbe* sp. without addition of antibiotics (3–9.5%).

When we added antibiotics to the cultures, we expected further reduction in survivorship in starvation treatments because larval bacteriophagy has been reported in other *Tisbe* sp. (Rieper 1982). However, unfed larvae exhibited elevated survival (30.0–42.7%) with antibiotics, suggesting that bacteria may have been having a pathogenic effect in food-absent larval environments. Other work in arthropods has also found that antibiotic treatment can improve survivorship in individuals experiencing starvation stress (Heys et al. 2018). Little is known about microbial parasites of copepods or their effects, but their potential to raise mortality is well-established (Mauchline 1998).

4.2. Larval development time

Larval development time decreased with rising temperature, as in other copepods (Webb & Parsons 1988, Hart 1990). We found both temperature and food to be important for larval development time, explaining 2.1 and 89.4% of variation (respectively) in Expt 2, while food alone explained 86.8% of the variation in Expt 1. The small effect of temperature compared to that of food level is somewhat inconsistent with previous work, which regards temperature as the most important factor for development rate in harpacticoid copepods (Hicks & Coull 1983, Williams & Jones 1994). However, the decreasing exponential relationships between development time and temperature generally observed in copepods means that

small temperature changes above 20°C may not appreciably decrease development time (Hart 1990). Further, within a given temperature, increased food availability is generally expected to further reduce development time (Hart 1990), until satiation. Nonetheless, development times under ad libitum feeding and starvation conditions (2.3–3.7 and 2.6–6.6 d, respectively) were comparable to those found in *T. battagliai* by Williams & Jones (1994), where satiated nauplii developed in 3–4 d and underfed (but not starved) nauplii developed in 4.5–6.2 d; and to those reported by Pinto et al. (2001) in *T. biminiensis* when fed ad libitum (2.05–2.61 d).

4.3. Juvenile size

Food supply explained 85.4% of the variance in post-metamorphic juvenile size, while the effect of temperature (1.8% of the variance) was marginally non-significant ($p = 0.09$) such that post-metamorphic size tended to be smaller at higher temperature. This temperature effect is consistent with previous work e.g. by Jamieson (1986) in 3 calanoid copepods. But the effect of temperature on juvenile length is typically not observed until late juvenile stages in copepods (Uye 1988, Escribano et al. 1997). We could not evaluate whether larval temperatures affected size at adulthood due to sparse data, owing to high larval mortality in starvation treatments.

Our results did not support recent work suggesting that, in addition to mediating development times, temperature may also mediate the costs of development. In their meta-analysis of temperature, development time and metabolic rate in 72 invertebrate species, Pettersen et al. (2019) found that a small (10%) increase in development temperature decreased the costs of development, which may result in smaller egg size. Offspring size has indeed been found to be smaller at higher temperatures in crustaceans (Efford 1969), including copepods (Kimoto et al. 1986). Parise (1975) also showed that eggs produced by female *T. holoturiae* at higher temperatures contained less energy than those produced by mothers reared at lower temperatures. We therefore expected larvae reared at 24°C to develop faster, survive better and be larger after metamorphosing (reflecting surplus yolk reserves from lower development costs being allocated to growth). However, we found that temperature had a marginally non-significant effect on size ($p = 0.09$), such that individuals reared at higher temperature were slightly smaller post-metamorphosis, suggesting that development costs were instead higher in the warmer environment. Pettersen et al. (2019) predicted that further increases in temperature beyond an upper optimum would instead increase the cost of development. Subsequent work across multiple phyla, including crustaceans, has supported this prediction (Marshall et al. 2020). In other words, we may not have observed reduced costs of development due to our high temperature treatment coming close to or slightly exceeding the temperature threshold for our study species. Pettersen et al. (2019) experimentally determined this threshold to be 24°C in *Bugula neritina*, collected from the same bay as our own study species (Port Phillip Bay, Melbourne, Australia) and close to the bay's maximum mean surface temperature of approximately 25°C. Assuming similar temperatures had been experienced by *Tisbe* sp. in the wild, we also tested for decreased development costs at 24°C (our high temperature treatment), which represented a small increase from their laboratory acclimation temperature of 20°C (our low temperature treatment). *Tisbe* sp. may still exhibit lower costs of development with a smaller increase in temperature (e.g. 22°C), but we expect larger increases in temperature (over 24°C) to further raise the cost of development.

5. CONCLUSIONS AND FUTURE DIRECTIONS

Marine invertebrates exhibit a rich diversity of life histories (Levin & Bridges 1995), providing excellent model systems for studying larval development strategies and how they are mediated by environmental gradients. However, intermediate larval nutritional strategies remain relatively unexplored in studies of the evolution of development (Allen & Pernet 2007). Facultative feeding has been considered rare in marine invertebrates, but the discovery of facultative feeding in species previously described as obligate larval feeders (including *Tisbe* sp.) or non-feeders suggests there may be more taxa whose larvae are able to feed but can complete development through metamorphosis without food. Reconsidering the prevalence of facultative feeding is further motivated by relatively recent models showing how intermediate larval nutritional strategies can optimise fitness, and from phylogenetic work confirming that these strategies are not necessarily ephemeral but can persist through evolutionary time. Facultative feeding might enhance resilience in the face of future increases in nutritional stress driven by local declines in marine phytoplankton populations, nutri-

tional quality and changes in phenology (Beardall et al. 2009, Ji et al. 2010). However, extrapolating from laboratory results to nature will also require a better understanding of how natural hazards interact with the prolonged larval phase experienced in food-poor environments. Optimal laboratory conditions insulate larvae from mortality — mortality rates for copepod nauplii in the field are typically very high, particularly due to predation (Santos et al. 2003, Vaughn & Allen 2010). Though challenging to directly replicate this kind of study in the wild, modelling could integrate laboratory nutritional stress experiments with field data to better elucidate the consequences of facultative feeding in natural systems (e.g. Pechenik & Levine 2007).

Future research could also address a number of results that were inconclusive in our study. First, we assumed that antibiotics eliminated all bacteria, but did not measure bacterial densities and consumption rates of bacteria by larvae. Quantifying these parameters would strengthen assumptions about starvation in the absence of provisioned food. Second, more work is needed on the impacts of larval starvation on adult performance in marine invertebrates with complex life histories, or lack thereof. Controlling for crowding (ideally by rearing juveniles individually) and ensuring a larger sample size (e.g. using a species exhibiting higher survivorship in the absence of larval food, or by allocating more larvae to starvation treatments) would strengthen any findings on latent effects in facultative feeders. Fitness consequences of larval starvation could also be measured more directly by considering lifetime reproductive output of survivors instead of first clutch size only. Finally, we agree with the 3 criteria proposed for facultative feeding by Allen & Pernet (2007), but wish to add a fourth. To demonstrate the functional relevance of facultative feeding, future work should test whether facultative feeding larvae are able to successfully reproduce in the absence of larval food.

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Appendix 1. Type III SS ANOVAs for larval performance in Expt 3. Survivorship was from hatching to the first juvenile stage, and development time was from hatching to metamorphosis. An asterisk (*) indicates statistically significant p-values. Survivorship increased in fed treatments, but contrary to Expt 2, warmer temperatures did not improve larval survival. Both warmer temperatures and feeding resulted in significantly lower development times

Response	Effect	df	Mean squares	*F*	p
Survivorship	Temperature	1	100.3	1.00	0.36
	Whole plot error	4	100.7	1.75	0.30
	Food	1	19825.4	344.10	<0.001*
	Food × Temperature	1	0.3	0.01	0.94
	Subplot error	4	57.6		
	Total	11			
Development time	Temperature	1	6.90974	209.68	<0.001*
	Whole plot error	4	0.03308	1.01	0.62
	Food	1	3.81376	116.83	0.06
	Food × Temperature	1	1.00273	30.72	0.11
	Subplot error	1	0.03264		
	Total	8			

Appendix 2. Mean ± SD egg size in mothers that had been unfed as larvae in Expt 3. Eggs were measured from the first clutch only. Due to high mortality in the larval stage, reproduction could not occur in the high larval temperature/low adult food treatment

	Mean egg size (µm)	
	Low juvenile food	Ad libitum juvenile food
Low temperature (larval)	79.8 ± 15.2	61.4 ± 9.9
High temperature (larval)	No data	53.3 ± 4.2

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