ORIGINAL PAPER

Benefits of intertidal development and large egg size in a marine toadfish

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Abstract

The environments in which offspring are reared can profoundly affect their development, growth, and survival. Certain specialized fish species deposit and sometimes care for their young in one of the Earth's most dynamic ecosystems: the marine intertidal zone. This tendency to deposit eggs in intertidal habitats rather than more stable coastal waters suggests intertidal conditions confer benefits to offspring that outweigh the risks parents face when spawning at the ocean's edge. Depositing eggs in the warmer intertidal zone might allow fish to produce and hatch larger eggs than would be possible in cooler subtidal waters, where development rates are much slower. We used two laboratory experiments and a field experiment to investigate how the benefits of intertidal development could vary according to egg size in the plainfin midshipman toadfish, *Porichthys notatus*. These toadfish breed along an intertidal gradient and down into shallow subtidal waters. We found that larger toadfish mothers produced larger eggs that hatched into larger young. Mothers laying these larger eggs also deposited them in warmer nests at higher tidal elevations, where the eggs can hatch into the largest juveniles with the highest survival rates. Juveniles that were raised in warm water in the laboratory (mimicking conditions in the high intertidal zone) experienced the highest survival rates during development and were also bolder and exhibited faster swimming speeds when fleeing a simulated predator. Taken together, these results suggest variation in propagule size can support divergent nest site choices among females, with females producing larger eggs benefiting more from depositing their eggs in warmer areas. Individual variation in propagule size could thus support niche partitioning by expanding the range of suitable conditions for nesting sites.

Introduction

Despite the dynamic conditions found in intertidal ecosystems, these habitats are used by fishes around the world as preferred spawning grounds (Horn and Gibson 1987). Intertidal spawning has arisen independently numerous

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times and does not appear to be the result of a gradual vertical migration up from deeper to shallower waters (Martin et al. 2004a; Martin and Swiderskif 2001). The prevalence and the success of this reproductive strategy beg the question: what draws fish to such a seemingly harsh environment to spawn? Early life mortality during egg and larval stages is the main determinant of abundance in fish populations (Bailey and Houde 1989; Houde 1989, 1997), and intertidal spawning is hypothesized to reduce egg and larval mortality by mitigating hypoxia and predation (Almada and Santos 1995; Ishimatsu et al. 2018). Eggs that develop in the intertidal zone experience periodic emersions that may enhance oxygen uptake (Strathmann and Hess 1999; Taylor and DiMichele 1983) and can reduce exposure to aquatic predators (Tewksbury and Conover 1987; Touchon and Worley 2015). Importantly, warm temperatures in the intertidal also accelerate embryonic development (Brown et al. 2021; Darken et al. 1998; Frank and Leggett 1981;

Jones 1972). However, depositing and fertilizing eggs in the intertidal zone exposes parents to predation by terrestrial animals and shore birds (Houpt et al. 2020a), and potentially to temperatures near or exceeding thermal tolerances (McArley et al. 2018), and also to aquatic hypoxia and air emersion (Bose et al. 2019; Horn and Riegle 1981; Sloman et al. 2008). Under what conditions then do the benefits of intertidal spawning for young outweigh the costs for parents and ultimately select for spawning in the intertidal zone?

High per-offspring investment and parental care are prevalent life history traits among intertidal-spawning fishes (Almada and Santos 1995; Ishimatsu et al. 2018), which, along with slow adult growth and long reproductive seasons, are hallmarks of an 'equilibrium' life history strategy (sensu Winemiller 1992). The equilibrium life history strategy is understood to be an adaptation for stable, density-dependent environments (Stearns 1992; Winemiller et al. 2015; Winemiller and Rose 1992). It is therefore somewhat puzzling that a majority of fishes that spawn in the stochastic intertidal zone exhibit equilibrium life history strategies that are typically associated with low environmental variability (Ishimatsu et al. 2018; Zeug and Winemiller 2007). Intertidal spawning could be an adaptation for equilibrium life history fishes to circumvent the principal limitation of producing large eggs in the marine environment—slow development. As large eggs generally take longer to hatch compared to small eggs (Gillooly et al. 2002; Kamler 2002), individuals hatching from larger eggs can require a longer period of parental care to ensure protection against predation and disease (Kolm and Ahnesjö, 2005). Raising and caring for eggs in the intertidal, where ambient temperatures are higher compared to nearshore subtidal waters, could increase the payoffs of providing care. In the warmer intertidal zone, embryonic growth and metabolism can accelerate, and yolk conversion efficiency can be enhanced, which would allow offspring from larger eggs to hatch earlier and bigger (Kamler 2002, 2008), and thereby enjoy higher juvenile survival (Einum and Fleming 2000; Pepin 1991; Segers and Taborsky 2011). However, larger eggs also require more oxygen (Kamler 2008), and dissolved oxygen levels decrease as water temperatures increase (Weiss 1970). Hence, periodic air emersion in the intertidal could afford enhanced oxygen availability to fish eggs developing in the warmer but more poorly oxygenated waters (Brown et al. 2021; Strathmann and Hess 1999). One aim of our study was to determine whether intertidal spawning enhances the success of fish by accelerating offspring development. A second aim of our study was to investigate the costs and benefits of emerging from a large versus a small egg. To address these aims, we studied a marine toadfish, the plainfin midshipman (*Porichthys notatus*), that nests on the rocky intertidal shores of western North America.

Female plainfin midshipman produce clutches of *c.* 200 spherical eggs per year that average around 6 mm in diameter, and they deposit them in nests attended by large "guarder" males (Arora 1948; DeMartini 1990). Plainfin midshipman guarder males excavate nest cavities under rocks, from which they vocalize their mating advertisement "hum" (Brantley and Bass 1994). These vocalizations attract females as well as small "sneaker" males to the nest and fecund females lay their eggs on the ceiling of the nest cavity while the guarder male and any attendant sneaker males release sperm to fertilize the eggs (Bose et al. 2018; Brantley and Bass 1994; Cogliati et al. 2014). Females and sneakers leave after spawning while the guarder males remain in the nest to care for their broods of young for up to 3 months (Cogliati et al. 2013). Female plainfin midshipman favor larger nests guarded by larger males (Bose et al. 2018; DeMartini 1988, 1990, 1991), but nest locations span a tidal gradient and thus vary considerably with respect to ambient temperatures and emersion durations that spawning and caring fish must endure. As fish nest higher up in the intertidal zone, the adults and their young will experience warmer and drier conditions during low tides (Fig. 1a and b). Nests at extreme ends of the tidal distribution can differ by up to 5 °C in daily average temperatures and by up to 5 h in daily emersion times (Bose et al. 2019; Brown et al. 2021). Previous observations on plainfin midshipman egg sizes describe a nearly twofold difference between the smallest and largest eggs (Arora 1948). If the temperature and oxygen benefits of developing in the intertidal are greater for larger eggs, then females laying larger eggs should experience greater reproductive success by depositing their eggs higher in the intertidal zone.

We hypothesized that, as observed in numerous other animal species, larger mothers and mothers in better condition would produce more eggs and larger eggs (Bonduriansky and Crean 2018; Kamler 2005; Wootton and Smith 2014). We further hypothesized that, all else being equal, mothers laying larger eggs would favor the warmest nests at the top of the environmental gradient to accelerate egg development. Using a field experiment and two laboratory experiments where we mimicked intertidal conditions, we tested five predictions that underlie our hypotheses: (1) egg size and fecundity increase with maternal body condition, (2) bigger eggs take longer to develop, (3) offspring hatching from larger eggs exhibit superior behavioral and physical traits—larger body size, higher boldness, and faster responses to stress, (4) warm temperatures and periodic emersion during incubation accelerate embryonic development and yolk conversion efficiency, and (5) larger eggs are laid in the wild at higher tidal elevations (where ambient temperature is higher).
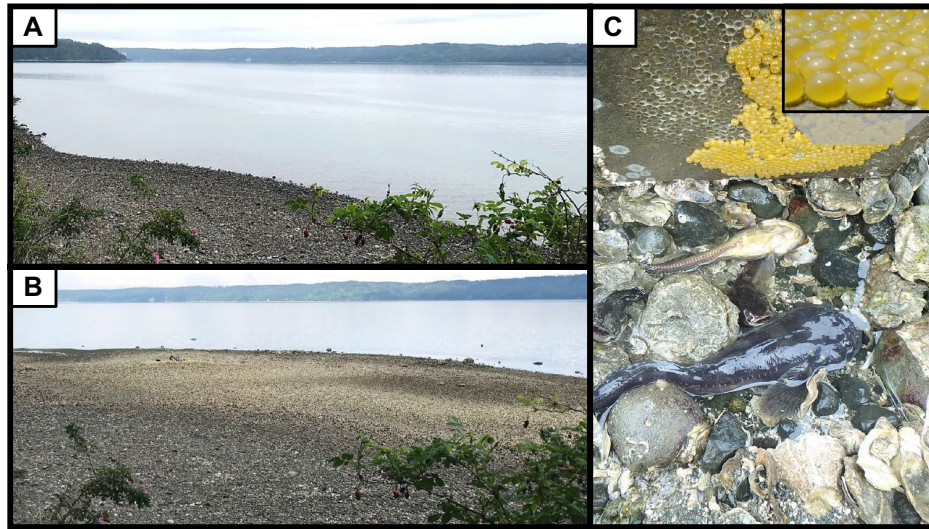
Fig. 1 A The field site in Dabob Bay, WA, USA, during the high-water phase of the 19 May 2018 spring tide (water height ≈ +0.61 m relative to local tidal charts). **B** The same field site during the low-water (receding) phase of the same spring tide (≈ −0.40 m relative to local tidal charts). Plainfin midshipman lay eggs in this intertidal zone, which is covered by water at high tide and uncovered at low tide. The higher a nest is up the beach, the warmer it gets and the longer it remains warm. **C** An overturned experimental tile nest with a brood of fresh eggs photographed during Experiment 1. The largest fish is the resident guarder male. The golden-bellied female is in the inverted spawning position. Between the guarder male and the female is a small cuckolder ("sneaker") male. The blow up photo of the eggs in panel **C** illustrates 1–2 days-post-fertilization (dpf) eggs with a white blastodisc that is visible on the surface of the yolk sac. Images captured by S. Balshine and N. Brown

Experiment 1: effects of egg size and simulated tidal conditions on developing offspring

This laboratory experiment investigated our first and second predictions that fecundity and egg size increase with maternal body size and condition, and that larger eggs take longer to develop.

Methods

During April and May of 2018, we collected plainfin midshipman guarder males and ripe females from breeding grounds in Ladysmith, White Rock, and Lantzville, BC, Canada (for detailed descriptions of these study sites see Bose et al. 2018; Brown et al. 2021; Cogliati et al. 2014), and transported the fish to the University of Victoria's outdoor Aquatic Unit, where they were housed in large (400 L) sex-specific tanks for 24–72 h prior to experimentation. We moved males and females from the holding tanks, measured their body masses (±0.01 g) and SLs (±1 mm), then placed male–female pairs into 175-L rectangular glass aquaria (L×W×H = 89×53×61 cm) supplied with free-flowing seawater (*c.* 13 ℃), lined with pea gravel, and equipped with brick structures to use as nests. We checked these tanks daily and allowed each pair up to three days to spawn before swapping the female for a new one from the holding tanks.

Males that had taken up the shelters as nests and successfully spawned with one female were allowed to do so again with a second female to improve the probability of spawning successes and minimize disturbances to each tank. Fish were weighed again immediately after they spawned. Using these methods, we collected fertilized batches of eggs from a total of 41 females who spawned with 21 males.

On the day after eggs were laid and fertilized, each brood was removed from the tank, and split by separating conjoined bricks that had formed the roof of experimental nests. Bricks with eggs were then distributed among different rearing environments that simulated the extreme ends of the tidal gradient across which plainfin midshipman nest. We chose to expose eggs to two temperatures 13 ℃ or 18 ℃ because in previous studies, we recorded daily average temperatures that differed by up to 5 ℃ between the lowest subtidal and highest intertidal nests (Brown et al. 2021). We also exposed eggs to either 4 h of air or to no air exposure because again in our previous studies, we observed that high intertidal nests experienced an average of 4 h of air emersion during spring tides (Bose et al. 2018, 2019). We used these field observations to inform a 2×2 crossed design of water temperatures and air emersion—either 13 ℃ or 18 ℃ seawater with either presence or absence of a 4-h daily emersion period. Each of the resulting four rearing environments was replicated in two 175-L rectangular glass aquaria (L×W×H = 89×53×61 cm), with up to 10 batches

of eggs on bricks in each rearing tank (8 tanks total; N per condition: 17 bricks in 13 ℃ with 4-h daily emersion, 18 bricks in 13 ℃ without emersion, 19 bricks in 18 ℃ with 4-h daily emersion, and 18 bricks in 18 ℃ without emersion). The eggs were always raised without any plainfin midshipman adults present to remove any confounding effects from parental care, but we placed a strong air supply near the eggs and we inspected the bricks daily, carefully removing any dead or infected eggs following the procedures outlined in Alderks and Sisneros (2013). Rearing tanks were supplied with free-flowing seawater that fully replenished each tank every ≈2 h. The warm seawater tanks were maintained at 18 ℃ by adjusting temperature controls on two 250-W aquarium heaters (EHEIM GmbH and Co. KG) in each tank; temperatures were monitored daily and the aquarium heater settings were fine-tuned as needed. Bricks with young in emersion treatment tanks were removed daily for a 4-h period (air temperature: mean ± SD = 18.4 ± 1.3 ℃; N = 3178 h). While emersion, bricks were covered with a plastic bin (to prevent light damage) and misted with seawater hourly (to prevent desiccation). Each brick was checked daily and photographed alongside a ruler every three days to track mortality and growth rates. For every partial brood of eggs, we recorded the day on which a new developmental stage was reached (or on which 50% of the eggs attained that stage if the transition exceeded 24 h). Plainfin midshipman young remain adhered to the nest substrate throughout their long embryonic, larval, and early juvenile development, only detaching from the surface once the yolk sac has been fully resorbed (Alderks and Sisneros 2013; Arora 1948). This feature of their ontogeny allowed us to also collect juvenile body size measurements from photographs taken 1–3 days before juveniles began detaching from their brick. A rater, who was unaware of the rearing environment, used *ImageJ* to measure the diameters of 20 newly laid eggs from each brood while a second rater, also unaware of the treatment, measured the SL of up to 20 juveniles on each brick in each treatment.

To investigate the effects of maternal traits on egg size, egg size variation, and fecundity, we fit multiple linear regression models to the initial (1 dpf) average egg diameters from each female's clutch, and to the coefficient of variation (CV) in egg diameters within each female's clutch. Predictor variables were female SL, female relative somatic body condition (RSC; see calculation in DeMartini 1990; Le Cren 1951), and fecundity (egg number). For each predictor, we calculated R^2_{LMG} to estimate its proportional contribution to the total R^2 (Grömping 2006; Lindeman et al. 1980). To investigate the effect of maternal traits on fecundity, we fit a Poisson generalized linear model (GLM) with a log link function to the fecundity data, and included female body length (SL) and female body condition (RSC) as continuous predictors.

The development duration and mortality rate data collected from the broods in this experiment were used to investigate how offspring growth rates and survivorship are affected by environmental conditions and at different levels of maternal provisioning (*Note*—some of these data have been analyzed previously, but to address different research questions; Brown et al. 2021). We ran three models to investigate three outcome measures of interest in relation to egg size: (1) total growth rates (number of days for all eggs to reach the free-swimming life stage; square root transformed), (2) survivorship (proportion of young from each replicate/brick that perished before reaching the free-swimming life stage), and (3) juvenile body lengths (SL achieved 1–3 days before young detached as free-swimming young from the bricks). Growth rates and juvenile body size data were fit with a linear mixed effects model (LMM) while mortality data were fit with a beta-binomial generalized linear mixed effects model (GLMM) with a complementary log–log link function. Fixed effects in all models were rearing water temperature (13 ℃ versus 18 ℃), emersion (4-h daily emersion versus no emersion), average initial egg diameter, and their three-way interaction. Mother and rearing tank identities were included as random intercepts in all models to account for repeated measures of bricks with eggs laid by the same mothers or reared in the same tanks. Brick identity was included as an additional random intercept in the juvenile body size model to account for repeated measurements of juveniles from the same brick.

Results

Fecundity in this laboratory experiment ranged between 19 and 230 eggs and egg diameters ranged between 4.0 and 7.0 mm; the variability (CV) in egg diameter within a female averaged 3.8% and ranged from 2.3 to 6.3% (N = 41 females). Across females, we observed a weak trade-off between egg size and number—with each 50-egg increase in fecundity, egg diameter decreased by approximately 2.3% (95% CI: 0.2–4.3%; model R^2 = 0.68; fecundity: R^2_{LMG} = 0.03, t_{35} = − 2.1, p = 0.04). Larger females and females in better condition laid larger eggs, with egg sizes increasing by approximately 10.8% (95% CI: 1.8–26.7%) with each 20% increase in female relative body condition, and by 24.2% (95% CI: 13.8–40.1%) with each 20% increase in female length (RSC: R^2_{LMG} = 0.04, t_{35} = 2.6, p = 0.01; SL: R^2_{LMG} = 0.61, t_{35} = 8.2, p < 0.001).

Larger juveniles hatched from larger eggs. On average, with each 1 mm increase in initial egg diameter, juvenile body length as measured by SL increased by approximately 2.7 mm (95% CI: 2.0–3.4 mm). Larger clutches were associated with females in better condition—fecundity increased by approximately 66 eggs (95% CI: 19–133 eggs) with each 20% increase in female relative body condition. However,

Fig. 2 Mediating effects of water temperature and emersion on the ▶ relationships between initial (Day 1) egg sizes and **A** offspring total development times, **B** survival to the free-swimming life stage, and (**C**) juvenile body sizes. Colored lines show LMM (panels **A** & **C**) and GLMM (panel **B**) predictions, and the shaded areas are their 95% CIs

fecundity was not clearly related to female body length (SL: est. [95% CI] = 23 eggs [−7 to 64 eggs], z_{34} = 1.5, p = 0.15; RSC: z_{34} = 3.0, p = 0.003).

Plainfin midshipman young took approximately the same time to develop within each rearing environment regardless of their initial egg size (temperature × emersion × egg diameter: $\chi^2(1)$ = 1.1, p = 0.29; Fig. 2a). Young raised in warm water (18 ℃) experienced a 39% (95% CI: 26–43%) increase in their chances of survival to the juvenile stage compared to young raised in cold water (13 ℃), regardless of emersion duration or initial egg size (temperature: $\chi^2(1)$ = 21.0, p < 0.01; temperature × emersion: $\chi^2(1)$ = 3.6, p = 0.056; temperature × emersion × egg diameter: $\chi^2(1)$ = 2.0, p = 0.15; emersion × egg diameter: $\chi^2(1)$ = 0.4, p = 0.54; Fig. 2b; Brown et al. 2021). When raised in 18 °C water with a 4-h daily emersion (conditions most closely mimicking nests in the high intertidal environment), juveniles grew approximately 2.8 mm (0.9–4.6 mm) larger than expected given their initial egg sizes; conversely, when reared under 13 ℃ water with no air exposure (conditions similar to subtidal nests), juveniles also grew approximately 2.7 mm (1.0–4.4 mm) larger than expected given their initial egg sizes (temperature × emersion × egg diameter: $\chi^2(1)$ = 18.2, p < 0.01; Fig. 2c).

Experiment 2: effects of simulated tidal conditions on juvenile behavior and physical performance

This laboratory experiment investigated our third and fourth predictions that larger initial egg sizes, warmer ambient temperatures, and periodic emersion leads to larger body size, greater boldness, and faster threat evasion in juvenile plainfin midshipman.

Methods

When juveniles reared in Experiment 2 (see above) finished resorbing the yolk and became free-swimming and capable of self-feeding, we continued to raise them in the same aquaria and under the same water temperatures that they had experienced during their embryonic development (13 ℃ or 18 ℃), but without daily emersion. This was an ecologically appropriate change in rearing habits because, in the wild, free-swimming juveniles immediately leave the nest
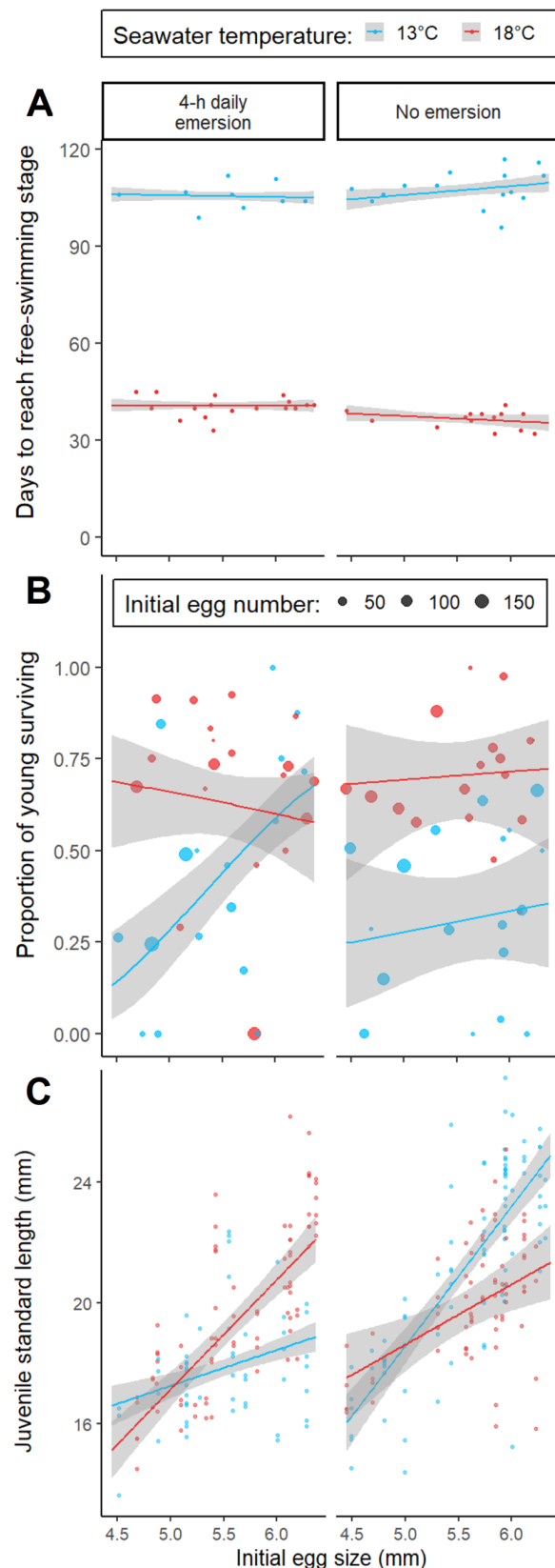
and move to subtidal waters where they are not air exposed (Robinson and Yakimishyn 2013). Once juveniles were free-swimming in the aquaria, we were unable to trace them back to their original brick, so inferences in the present experiment could not be traced back to individual mothers. Water temperatures in the 18 ℃ tanks were maintained as described in Experiment 2 (see above). Juveniles in all tanks were fed 2 ml of SELCO-enriched live adult brine shrimp daily on a per-fish basis supplemented with wild-caught marine amphipods (*Hyale spp.*). Across the tank replicates, the maximum age difference between juveniles was 16 days in the 18 ℃ tanks and 29 days in the 13 ℃ tanks.

For each tank, we began physical tests once ≈50% of the bricks had shed free-swimming juveniles. Two batteries of tests were administered to evaluate boldness, activity levels, and fast-start swimming speed, all of which are important behavioral correlates of juvenile fish survival (Biro et al. 2005; McCormick et al. 2018). The first battery of tests to assess boldness in the newly free-swimming juveniles was carried out between 9 August and 29 November 2018. Tests were conducted in three identical 10-L glass aquarium tanks (L×W×H=30×20×20 cm) lined with sand and filled with water from the juveniles' source tank. The purpose of testing juveniles under their exposure water temperature was to mitigate any behavioral alterations that might have resulted from requiring young fish to adapt to a new water temperature. As each test was quite short (20-min), water temperatures and DO were not actively maintained during testing; measurements before and after showed that water temperature differences varied from -0.4 to 0.6 ℃ (post – pre-test) and DO differences varied from -0.6 to 0.1 mg•L^{-1}. To minimize interference from external stimuli and standardize lighting conditions, we covered the tanks with an opaque blind, while two 300-lm LED lights (Blackfire Waterproof LED Clamplight, BBM905) illuminated the tanks from within. A small video camera was mounted above each tank to record the tests. Using a handheld dipnet, fish were captured at random from rearing tanks, placed into 12×6 cm (height×diameter) cylindrical start chambers filled with tank water (Figure S1), and transferred to the experimental tanks in these start chambers. Fish were allowed 10 min to acclimate and recover following transport in these start chambers which were then remotely opened and the latency of each fish to exit the start chamber was recorded for up to 600 s. Each fish was tested thrice using the exact same procedure but in three different tanks to minimize the risk of one testing arena or its location skewing the results. Fish were allowed 15 min between tests to recover from transfer stress. After the final test, we measured the body masses (± 0.01 g) and SL (± 0.1 mm) of each fish.

From 11 to 16 December 2018, we conducted a second battery of behavioral tests on older juveniles (2–3 months of age) to measure their activity levels and fast-start swimming

response to threats. We had only enough surviving juveniles of this age to compare performances between those that were reared in seawater held at 13 ℃ or at 18 ℃. Each fish was tested twice with three days between tests. Tests were based on the procedure used in McCormick et al. (2018) and were carried out in a small glass aquarium (identical to those used in the preceding tests on newly detached young) filled with *c.* 16 ℃ seawater to 6-cm depth. We chose to test these older juveniles in an intermediate water temperature to ensure swimming speeds and activity levels were not confounded with water temperature in these tests. Fish were first placed into a start chamber (6-cm diameter cylinder capped at both ends) within a 15 cm diameter circular test arena (Figure S1). A trap door to the start chamber was then remotely opened and each focal fish was given eight minutes to emerge. Fish that did not emerge in eight minutes were gently pushed out of the start chamber remotely by engaging a pulley system that advanced a panel enclosing the rear end of the start chamber (Figure S1). Once in the arena, fish were given 30 min to move around freely, during which time we measured their swimming activity. We recorded the duration of time spent swimming in the first 3 min of the trial, and then again for 10 s at 10, 20, and 30 min into the trial. We then tested each fish's fast-start ("C-start") swimming response to a simulated predator attack, where an experimenter used a glass rod to tap the focal fish's tail (Domenici and Blake 1997; Figure S1). A rater, unaware of the treatment group, used the *Tracker* software (version 5.1; Brown and Cox 2009) to calculate fast-start swimming velocities (mm s^{-1}) from measurements of the elapsed time and total distance covered by each fish during the first two axial bends of its tail (a critical period for avoiding predator attacks; Domenici and Blake 1997; Webb 1976). We measured fish body masses (± 0.01 g) and SLs (± 0.1 mm) after each test.

We investigated how rearing environment and body size affected juvenile boldness (latency to fully exit the start chamber) in the first battery of tests when the fish were newly detached. Because 17 fish did not exit the start chamber on their own accord during at least one of their three tests, we employed a two-step *hurdle model* to analyze the data (Steel et al. 2013). In the first step, we used a binomial GLMM to model whether fish exited the start chamber or not. In the second step, we used a log-LMM to model latency to exit the start chamber for only the fish that did so during the 600 s trial period. In both models, fixed effects were rearing environment (water temperature, emersion, and their interaction) and the fish's body size (SL). We controlled for fish size to account for the variation in fish ages. We included nested random intercepts for fish ID within rearing tanks to account for repeated measurements of individual fish over three tests, and of multiple focal fish from each rearing tank.

In the second battery of tests on the older juveniles, we investigated how body size and rearing temperature

affected juvenile activity levels and fast-start swimming speeds. Water temperature, test order (first or second), and the fish's body length (SL) were again fixed factors, and fish identity and rearing tank were included as random intercepts in all models. Duration of time spent swimming was converted to a proportion of total observation time and modeled with a binomial GLMM. Fast-start swimming velocities met normality assumptions and were modeled using an LMM.

Results

In the first battery of tests, newly hatched juveniles raised in 18 ℃ water exhibited bolder behavior compared to those raised in 13 ℃ water by exiting their start chambers approximately 2.5 s (95% CI: 1.5–4.3 s) earlier (LMM; water temperature: $\chi^2_1 = 4.5$, $p = 0.03$; emersion: $\chi^2_1 = 0.7$, $p = 0.41$; SL: $\chi^2_1 = 0.01$, $p = 0.92$; Fig. 3a). In the second battery of physical performance assay tests with older juveniles, activity duration increased with body size—fish spent 2.7% (95% CI: 0.4–4.9%) more time swimming in the open arena for every 1 mm increase in body length—but rearing water temperature did not significantly affect swimming activity levels (GLMM; SL: $\chi^2_1 = 4.5$, $p = 0.03$; temperature: $\chi^2_1 = 2.4$, $p = 0.12$). However, the older juveniles that were raised in warm water swam 90 mm s^{-1} (95% CI: 1–179 mm s^{-1}) faster than older juveniles raised in cold water, while body size did not significantly correlate with swimming speeds (LMM; rearing water temperature: $\chi^2_1 = 4.4$, $p = 0.04$; SL: $\chi^2_1 = 1.7$, $p = 0.20$; Fig. 3b).

Experiment 3: distribution of parental traits and egg sizes along a natural tidal gradient

This field study investigated our fifth prediction that larger eggs would be selectively deposited in warmer environments higher in the intertidal zone.

Methods

Data were collected from May to July in 2018 and 2019 at a plainfin midshipman breeding ground in Dabob Bay, Washington, USA (47°76′N, 122°86′W). At this site, we constructed artificial nests along tidal elevation contours, demarcated by the waterline during receding tides. Nests were shallow pits in the substratum that we covered with concrete garden tiles (929 cm^2), which plainfin midshipman readily use for spawning and parental care (Bose et al. 2018; Brown et al. 2021; Cogliati et al. 2013; Fig. 1c). In 2018, 20 nests each were placed along four contours that were demarcated on local tidal charts by their varying water levels as having different tidal heights. We classified these contours as "high intertidal" (− 0.03 m), "middle intertidal" (− 0.40 m), "low intertidal" (− 0.61 m), and "subtidal" (− 1.22 m). Digital temperature loggers (± 0.1 ℃ Onset HOBO Pendants and ± 0.5 ℃ Thermochron iButtons recording at one temperature measurement per hour) were deployed in 10 nests along each intertidal contour and in three subtidal nests. Analysis of these data showed that during our study, between 21 May and 10 July 2018, ambient temperatures were similar between high and middle contour nests, but decreased ≈0.3 ℃ between middle intertidal and low intertidal nests, and decreased additionally by 1 ℃ between low

Fig. 3 **A** Juvenile boldness scores during boldness tests. Each point shows the measurement of one juvenile in one test (each juvenile completed three tests; $N = 77$ fish). A total of 17 fish did not exit their start chamber in at least one of their c. 600-s tests and these tests are represented by empty (unfilled) circles. **B** Fast-start swimming speeds as a function of a juvenile's rearing tank water temperature. Each colored point shows the average velocity of one fish across the two testing rounds. Black points and lines show treatment medians and interquartile ranges, respectively
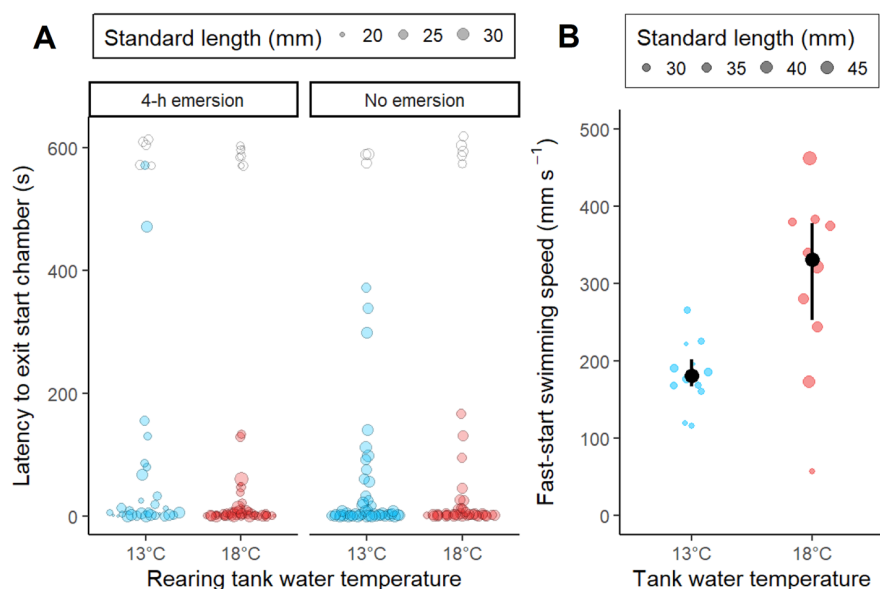
intertidal and subtidal nests (Brown et al. 2021). In 2019, 30 nests each were constructed along the middle intertidal and subtidal contours, and 38 additional nests were constructed at various intertidal elevations ranging from −0.69 to −0.12 m (these extra nests were set up for another experiment; Woods et al. 2022).

In both years, we checked the artificial nests once every 2–3 days. If a guarder male was discovered without a brood of eggs, we left the nest alone and checked it again the following day and so on. When we discovered a guarder male with a brood of eggs, we photographed the brood of eggs alongside a ruler for later image analysis of egg sizes. We also captured the male and any females or sneakers present in the nest and measured their body masses (±0.01 g) and standard lengths (SL, ±1 mm) before returning the guarder male back to the nest; any sneaker males or females found were released into the ocean. To investigate how female body sizes varied along the tidal gradient, we fit the female SL data with an LMM. Fixed effects were tidal elevation and day of the year. We included random intercepts for year (2018 or 2019) with nest ID nested within year to account for repeated measurements from some nests on different days in the breeding season and from nests sharing roughly the same location between years.

A rater unaware of each nest's elevation used *ImageJ* (Schneider et al. 2012) on the digital photos to measure diameters (±0.1 mm) of up to 20 randomly selected freshly laid eggs from each clutch within nests. We further categorized these counts of fresh eggs into two egg development stages: 1–2 days-post-fertilization (dpf) were characterized by the presence of a white blastodisc that is visible on the surface of the yolk sac while 3–5 dpf eggs were characterized by the formation of a thin crescent-shaped blastoderm (Brown et al. 2021). In total, we measured 7750 new eggs from 390 clutches. The diameter of the egg was used because plainfin midshipman eggs are approximately spherical (DeMartini 1990; Fig. 1c). Investigative quantile–quantile plots revealed that these egg diameter data exhibited greater symmetrical variability around the mean than assumed in an LMM, so we instead used a robust LMM, which reduces the leverage of outlier observations in proportion to their extremity, to model the egg diameter measurements (Davies 1993; Koller 2016). Fixed effects in this model were tidal elevation, developmental stage (1–2 dpf or 3–5 dpf eggs), and day of the year. We included nested random intercepts for brood ID within nest ID within year to account for measurements from multiple broods from the same nest, to account for the fact that nests were sometimes measured repeatedly on different days, and to account for data collected from nests in 2018 and 2019, respectively. As *p* value calculations are not well-defined for robust LMMs (Koller 2016), for these analyses, we provide parameter estimates and 95% confidence intervals only.

Results

Deposited clutch sizes in this field study ranged from 16 to 327 eggs and egg diameters ranged from 3.3 to 8.3 mm ($N = 99$ females). Artificial nests in the field spanned an elevation gradient of 119 cm (vertical). Egg diameters increased approximately 0.1 mm (95% CI: 0.1–0.2 mm) with each 50-cm increase in tidal elevation (RLMM; Fig. 4a), but fecundity did not vary significantly with respect to tidal elevation (LM of fecundity on elevation; $p = 0.74$). Female body sizes declined by approximately 9 mm (95% CI: 3–14 mm) with each 50-cm increase in tidal elevation (LMM on SL; elevation: $\chi^2_1 = 10.2$, $p = 0.001$; day of year: est. [95% CI] = −1 mm [−2 to 0 mm] per week; $\chi^2_1 = 2.3$, $p = 0.13$; Fig. 4b). However, this relationship between female size and tidal elevation should be interpreted cautiously as it was strongly influenced by seven females captured in subtidal nests (Fig. 4b). All intertidal females were captured by hand on dry land when nests were emersed by the recently receding tide and therefore we could be sure at low tides that no females were missed. In contrast, subtidal nests were never emersed and subtidal females were captured by snorkelers using a hand net to cover the excavated cavity underneath experimental tiles (see details in Brown et al. 2021). Therefore, smaller females (which could be nearly
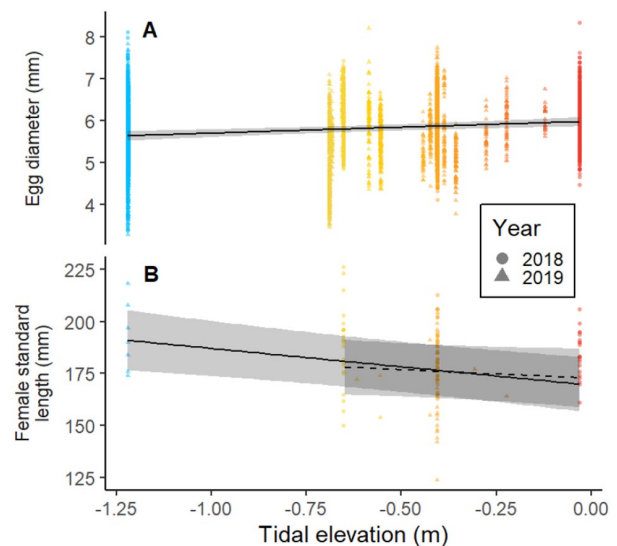
Fig. 4 **A** Egg diameters measured from 390 photographed broods and plotted as a function of tidal elevation. **B** Standard lengths of female plainfin midshipman fish plotted as a function of the tidal elevation from which they were captured. Black lines show fitted model predictions, and the shaded areas show the 95% CIs. The dashed line in panel B shows the regression fit when subtidal females are excluded. Points are colored according to tidal elevation as a visual aid with blue indicating subtidal nests, yellow the low contour nests, orange the medium contour nests, and red the high contour nests

half the length of the largest females; Fig. 4b) could have evaded capture more easily than larger females by swimming through small gaps between the net rim and the periphery of the cavity. It is therefore possible that smaller fish could have escaped. Thus, our method for capturing females in the subtidal could have been biased toward larger fish. Considering this possible bias, we reran the models excluding data from the subtidal females and when we did so female size did not correlate significantly with tidal elevation (LMM on SL: elevation: est. [95% CI] = − 4 mm [−3 to 12 mm], $\chi^2_1 = 1.2$, $p = 0.28$; Fig. 4b).

Discussion

When deciding where to raise their young, parents select among rearing sites with varying benefits and costs (Refsnider and Janzen 2010). The objective of our study was to determine how the marine intertidal zone benefits developing offspring, and whether these benefits vary according to egg size in the plainfin midshipman. In agreement with our first prediction, egg size increased with both female body size and body condition, while fecundity increased with female body condition but not body size. However, we found no support for our second prediction that larger eggs would take longer to develop; development times were similar across eggs of all sizes within temperature and emersion treatments. In agreement with our third prediction, larger juveniles hatched from larger eggs, and these larger juveniles exhibited higher boldness, activity levels, and faster swimming speeds in open arena tests. However, as juveniles were tested for boldness in the same temperatures under which they were raised, we cannot rule out a possible effect of testing water temperature on boldness. Our fourth prediction was partly supported; eggs in warm water with regular emersion appeared to have enhanced yolk conversion efficiency, producing juveniles that were large given their initial egg sizes. Rearing in warm water was also associated with faster embryonic development, higher survival to the juvenile life stage, and superior swimming speeds, whereas regular emersion during development had no clear effect on development speed, offspring survival, or the behavior of juveniles. In agreement with our fifth predication, we found that, in the wild, larger eggs were deposited in the higher tidal elevation nests that were also the warmer environments.

Considering the development and behavioral benefits of rearing in warmer water, our results indicate that high intertidal conditions may confer extra benefits to larger eggs. Our results showed that yolk conversion efficiency was enhanced under warm water with periodic emersion, with larger young emerging. However, we also observed an equal benefit of offspring growing larger for their initial egg sizes when raised in cold water without emersion. These results suggest

that eggs could receive the benefit of enhanced yolk conversion efficiency in nests located at ether extreme of the tidal gradient. However, given that rearing in cold water more than doubled development times and decreased survivorship by 85% relative to warm water (Brown et al. 2021), rearing in warm water with emersion clearly provided the best conditions for development in the laboratory. While all young stand to benefit from enhanced yolk conversion efficiency by hatching into heavier juveniles that will be safer from predation (Beacham and Murray 1985; Jones 1972; Kamler 2008), large eggs may be better adapted to conditions in higher intertidal nests compared to smaller eggs because large eggs are more resilient to hypoxia and desiccation (Einum et al. 2002; Strathmann and Hess 1999). Laying large eggs in high intertidal nests might thereby allow females to produce the largest possible young while buffering these young against the mortality risks associated with extreme conditions, and while avoiding the prolonged development times observed in cooler subtidal nests (Brown et al. 2021).

Relative to other marine fishes, whose egg diameters typically range from 1.0 to 2.2 mm (Wootton and Smith 2014), plainfin midshipman eggs are exceptionally large at *c.* 6 mm diameter. Also, egg sizes in our study were highly variable among females, with a more than twofold difference between the smallest and largest egg diameters observed in the wild (3–8 mm). Female body length accounted for 16% (field) to 61% (laboratory) of this variance in egg size. These large differences in egg sizes between mothers may drive females to select nest sites that are better suited for the size of their eggs. Accordingly, we expected to capture larger females high up in the intertidal, but instead observed no clear relationship between female body size and tidal elevation. The lack of a clear relationship between female body size and elevation may be partly due to our sampling design; we captured females only during low spring tides when the nests were easily accessible. However, females are likely to leave nests as the tides recede and many females also enter nests to spawn during the neap tide cycle when most nests are not emersed (Bose A. and Brown N. unpublished data). Future studies should focus on more representative sampling across the lunar cycle to get a more complete picture of female spatial demographics.

Females consider several different factors when determining where to deposit their eggs, and selecting nest sites based on a matching elevation and egg size could be a lower priority. Mate quality can be the cardinal determinant of female reproductive success, in species such as the plainfin midshipman, where males provide sole parental care (Bernardo 1996; Bose et al. 2016; Crean and Bonduriansky 2014; Refsnider and Janzen 2010; Wong and Candolin 2005). Plainfin midshipman males care for broods over a *c.* 2-month development period by clearing debris from the eggs, fanning them, and guarding them against predators (Brantley and

Bass 1994; Cogliati et al. 2013; Woods et al. 2022). Additionally, plainfin midshipman guarder males invest heavily in reproductive organs, known as accessory glands, that contain anti-microbial compounds that protect eggs against bacterial or fungal infections (Miller et al. 2019; Pepler et al. 2021). Larger males typically provide more care and might also have energy reserves to sustain a longer parental care duration, increasing the survival of young (Suski and Ridgway 2007). Previous studies have detected strong female preferences for larger plainfin midshipman males (Bose et al. 2018; DeMartini 1988), which indicates that mate quality is likely a strong driver of nest site choices in the species. Females can thus exhibit indirect environmental selectivity through their preference for high quality mates (Candolin and Voigt 2001). Since larger plainfin midshipman males tend to nest lower in the intertidal zone (Brown et al. 2021), females may forego the potentially smaller fitness benefits associated with laying larger eggs in warmer (higher) nests to accrue more substantial fitness benefits associated with having their eggs tended by a superior larger male.

It is also possible that simply laying eggs anywhere in the intertidal region is "good enough" to attain the fitness benefits associated with warmer incubation temperatures—*i.e.,* any benefits that accrue for a particular egg size could be overridden by more universal benefits of getting warmer development conditions (Morjan and Janzen 2003). For example, fish predation on eggs of the pantless treefrog (*Dendropsophus ebraccatus*) poses such a severe threat to offspring survivorship that mothers will risk embryo desiccation and deposit their eggs on emergent plants rather than in pools with predatory fish (Touchon and Worley 2015). For these treefrogs, the hydration benefit of incubating eggs underwater is overshadowed by the benefit of isolation from aquatic predators, and female oviposition preferences have evolved accordingly. Similarly, any benefits plainfin midshipman mothers might receive from fine-tuning their oviposition site preferences to match their egg sizes could be overshadowed by severe mortality risks that are associated with particular tidal elevations, such as desiccation when high up on the beach or the continuous pressure from aquatic egg predators when low down in the subtidal (Brown et al. 2021; Tewksbury and Conover 1987). It is presumably costly for females to spawn higher in the intertidal zone. Fish in higher nests are subjected to higher rates of avian predation (Houpt et al. 2020a) and more frequent and prolonged emersion periods that bring extreme temperatures and oxygen limitations (Bose et al. 2019; Brown et al. 2021; Houpt et al. 2020b). Compared to guarder males, females (who remain in the intertidal for only 1–2 days) are less adapted to withstand these harsher nesting conditions. Females have a lower tolerance for buildup of plasma lactate and a lower oxygen carrying capacity (Craig et al. 2014). Thus there may be greater mortality risks to females when spawning in higher intertidal nests where emersion periods are more frequent and longer, and temperatures are higher; these risks to females may outweigh the benefit to larger eggs of developing in this warmer environment.

The results of this study, in combination with our previous work, demonstrate that conditions found in the intertidal environment confer numerous benefits to plainfin midshipman young compared to environmental conditions characteristic of the nearshore subtidal. Warmer temperatures accelerate embryonic development rates, and enhance juvenile boldness, activity levels, and swimming speeds, traits that in other fishes are associated with higher survivorship (Brown et al. 2021; McCormick et al. 2018). Furthermore, our laboratory data indicate that mothers with larger eggs could improve yolk-conversion efficiency by laying eggs higher in the intertidal zone. In support of this idea, we detected an increase in egg sizes with increasing tidal elevation in the wild. If there is substantial variation in optimal nesting conditions according to egg size in the plainfin midshipman, the resultant female preferences for nests high up in the intertidal zone could work to relax the intensity of male–male competition and support the maintenance of phenotypic diversity among males (Takahashi et al. 2014). Interspecific differences in optimal nesting conditions could be an important driver of niche partitioning in landscapes when nesting habitat is limited, as is the case for plainfin midshipman (DeMartini 1991; Martin et al. 2004b; Vickruck and Richards 2012). A niche partitioning phenomenon could arise from intraspecific differences in optimal nesting conditions. If offspring size variation supports divergent female nest site preferences, then the smaller plainfin midshipman males that cannot outcompete the larger males found in low intertidal nests (Brown et al. 2021) could still gain some reproductive success by taking up less-competitive nests higher up in the intertidal. Hence, intraspecific variation in rearing site preferences might drive and maintain phenotypic diversity more generally.

Declarations

Conflict of interest We declare no financial or non-financial conflicts of interest.

Ethical approval Collection and animal housing procedures employed in our experiments were reviewed and approved by the McMaster University Research Ethics Committee (AUP: 18-01-02) and the University of Victoria Animal Care Committee (AUP: Juanes-2017–003), in accordance with guidelines set forth by the Canadian Council on Animal Care (Olfert et al. 1993). Our practices were subject to regular review by the University of Victoria Animal Care Committee during experimentation.

Consent to participate N/A.

Consent to publish N/A.

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