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Marine and Freshwater Research

Supplementary Material

Reproductive phenology and the influence of temperature in two sympatric New Zealand freshwater mussel species

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Context

Two separate laboratory experiments were conducted to support field observations of temperature regimes and for ADD calculations. For the first experiment, minimum thermal tolerance was tested in gravid *E. menziesii* and *E. aucklandica* to determine at what temperatures reproduction was likely disrupted and abortions of unviable larvae occurred. Procedures were similar to an earlier study (Melchior 2017) that examined differences in larval release timing in *E. menziesii* held at constant laboratory temperatures of 8, 12 and 18°C. Based on those findings, that early larvae of *E. menziesii* were aborted at 8°C whereas at 12 and 18°C mussels released mature glochidia, a narrower gradient of constant water temperatures of 9, 10 and 11°C was used to refine minimum thermal thresholds for *E. menziesii* compared with *E. aucklandica*.

Methods

During the austral mid-summer (December 2019) at Ohautira Stream, 15 gravid (Stage 3-4) females of each species were collected for the first experiment and kept separately in two aerated buckets containing stream water (17°C) and substrate for transport to the laboratory to examine species-specific minimum thermal thresholds. Individuals were randomly divided into three static temperature treatments (n = 5 of each species at 9, 10 and 11°C; light/dark cycle of 16/8 hours), and held separately in 2.5-L glass aquaria containing 3-cm silica sand and oxygenated with aerators. Individuals were fed every other day with a 2:1 algae mixture using Reed Marine shellfish diet and *Nannochloropsis* (Reed Mariculture, Campbell, CA, USA). All mussels were acclimated to the same thermal regime of 12°C for 7 days before decreasing temperatures to three randomly assigned treatments by moving the aquaria to separate controlled temperature rooms set at the corresponding temperature. Observations of glochidia release were recorded daily. If glochidia were released, they were removed, counted, and analysed for viability, characterised by (i) the presence of hooks on opposing valves, (ii) translucent valves, free of their vitelline membrane, and (iii) rapid opening and closing of the glochidia valves viewed under a binocular microscope (40× magnification).

The second laboratory trial was undertaken to test for maximum thermal thresholds for the first release of glochidia and peak release, and to detect any differences in the influence of temperature on the timing of larval release between each species. Here, thermal limits of initiation in larval release were estimated using a dynamic thermal threshold method (Lutterschmidt and Hutchison 1997) in which individuals of both species were exposed to a constant increase in temperature. For this experiment, 6 gravid females (Stage 4; see Melchior *et al*. 2021) of each species were collected in summer following the procedures described above. Individuals were acclimated to laboratory conditions for 7 days at 12°C with a 16/8 hour light/dark cycle (see plate). That temperature was chosen as this was a temperature at which unionids are known to delay brood timing (Melchior 2017). Individuals were held separately, monitored daily for potential glochidia release, and fed as in Experiment 1. At the onset of the trial, temperatures were increased at a rate of 1°C day^{-1} until glochidia release by each mussel peaked. The threshold for larval release was then determined as the mean thermal point at which individuals had

released at least (i) 100 viable glochidia for *E. menziesii*, or (ii) one conglutinate containing viable glochidia in *E. aucklandica.* ADD required for glochidia release were calculated for each species.

Fig. S1. Laboratory trial set-up for testing thermal tolerance in gravid *Echyridella menziesii* and *E. aucklandica*.

Results

Minimum temperature trial

The 9°C treatment resulted in premature glochidia release in both species, with the expulsion of individual unviable larvae in *E. menziesii* (released larvae per individual, mean ± s.d. = 1063 ± 973.6, n = 5, range 175–2785) and conglutinates containing closed (unviable) larvae in *E. aucklandica* (mean number of released conglutinates per individual = 89 ± 93.6, n = 5, range 3–240). Larvae of both species were aborted within the first 5 days of the 9 °C treatment, with no significant differences in the timing of premature release between species (t_4 = 0.172, P = 0.87). At the end of the experiment, no major brood releases were observed by individuals of both species exposed to 10 and 11°C treatments, although there was minor release by one *E. menziesii* held at 10°C, which occurred 18 days into the experiment. Brood inspections after 20 days of exposure to low temperatures confirmed no or minimal larval release at these treatments, whereas within the 9°C treatment only empty or half empty broods were found. Given that 10°C was the threshold temperature below which premature release occurred in this study (except for one occurrence), and considering the glochidia abortions reported at 8°C by Melchior (2017), 10°C was chosen as the minimum temperature to be used for the calculation of ADD when exploring developmental thresholds for both *E. menziesii* and *E. aucklandica* among sites.

Maximum temperature trial

During the acclimation period at a constant 12°C, one individual *E. menziesii* was observed to release part of its brood and was therefore excluded from the remainder of the trial. In *E. aucklandica*, glochidia release occurred at the first instance of temperature increase by 1°C at

13°C (equivalent to 97 ADD including the 7-day acclimation period), with 3 out of 6 individuals observed to have released conglutinates. Peak release (6 out of 6 individuals releasing viable conglutinates) occurred between 17 and 18°C (159–177 ADD), however, conglutinates were still gradually being released at 21°C (237), 8 days after initial release. The mean number of conglutinates released per individual was 164.8 ± 65.3 (range 91–241). Viability of released glochidia remained high (>85%) with each temperature increase.

In *E. menziesii*, peak release occurred at 18°C (177 ADD) (5 out of 5 individuals) with viable glochidia continuing to be released for 9 days post initial release at 22°C (259 ADD). Mean number of glochidia released at the peak was 2178 ± 2256.3 (range 1000–6200). Mean viability in released glochidia at the first increases in temperature was low, but gradually increased at 17°C (See Fig. S2 below), indicating possible stress release within the first days rather than the reaching a natural maximum threshold for larval release.

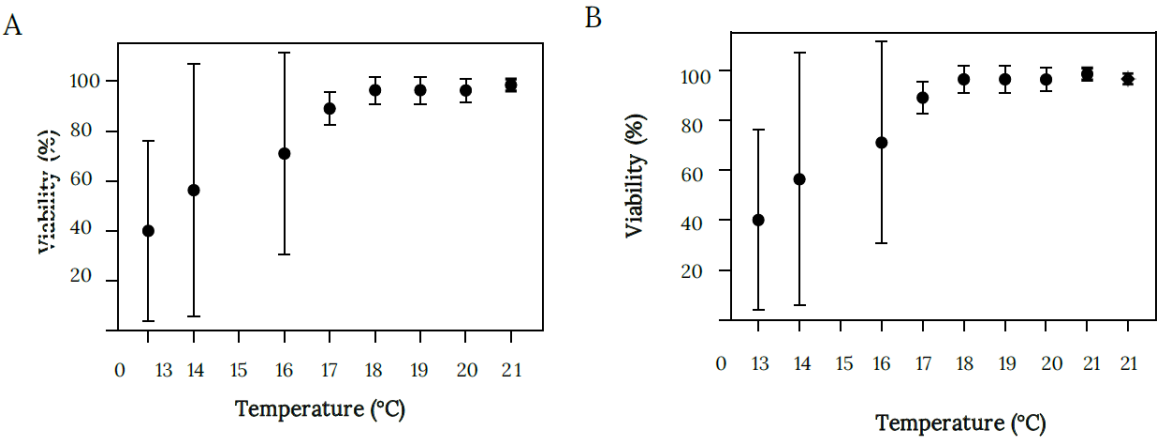

Fig. S2. Mean viability for *Echyridella aucklandica* (A) and *E. menziesii* (B) at each temperature increase throughout the laboratory trial.

Table S1. Physicochemical data showing monthly temperature, dissolved oxygen, specific conductivity among sites with temperature ranges in parentheses (refer to Table 3 for annual summaries).

		Month (2018-19)												
Site	**Parameter**	**March**	**April**	**May**	**June**	**July**	**August**	**September**	**October**	**November**	**December**	**January**	**February**	**March**
Ohautira	Water temperature (°C)	16.8±0.3	14.5±2.1	12.5±1.7	10.2±1.7	9.8±1.3	10.4±0.8	11.5±1.5	12.4±1.5	14.7±1.5	16.4±1.2	18.3±1.5	18.9±1.5	17.1±1.1
		(15.6 –18.1)	(11.6–17.4)	(6.9–15.9)	(6.8–13.7)	(6.4–13.7)	(8.5–12.5)	(8.6–16.1)	(9.2–14.7)	(10.6–18.2)	(12.6–20.5)	(15.2–23.2)	(13.9–22.1)	(14.2–19.2)
	Dissolved oxygen (%)	111.8	117.4	99	110.4	105.1	95.5	102.9	103.2	97	110.7	104.8	101.8	108.6
	Dissolved oxygen (mg L^{-1})	10.8	12.4	11.1	13	12.2	9.7	11.4	11.2	10.3	10.9	10.1	9.4	11
	Specific conductivity (µs cm^{-1} @ 25°C)	157	154	137	151.4	106.1	130	121	146	152.1	129	138.6	110.4	160.1
Kahururu	Water temperature (°C)	17.6±0.3	15.5±1.4	13.8±1.7	11.1±1.7	10.7±1.3	11.2±0.8	12.7±1.2	13.5±0.9	15.5±1.1	17.8±1.1	19.6±1.3	20.2±1.3	17.4±1.2
		(17.2–18.2)	(12.7–18.3)	(7.9–16.7)	(7.6–14.6)	(7.3–13.5)	(9.4–13.5)	(10.5–15.2)	(15.4–21.8)	(13.1–18.4)	(15.4–21.8)	(16.1–22.7)	(17.3–24.2)	(14.0–20.7)
	Dissolved oxygen (%)	108	80.5	94.3	99.7	100.6	93.5	96.5	91.7	106.7	104.1	101.7	88.2	69.5
	Dissolved oxygen (mg L^{-1})	10.3	8.4	10.2	11.6	11.6	10.5	10.6	9.2	10.3	9.9	10.7	8.7	6.9
	Specific conductivity (µs cm^{-1} @ 25°C)	165	166	124.2	161.9	130	150	147	154	154	140.2	153.4	160.8	181.6
Pakoka	Water temperature (°C)	18.4±1.9	14.9±1.8	13.4±1.5	10.7±1.6	10.7±1.2	11.1±0.8	12.1±1.7	13.1±1.3	15.0±1.8	17.6±1.7	18.9±1.8	19.7±2.0	17.8±1.6
		(14.5–24.4)	(9.9–18.9)	(8.1–17.4)	(6.9–13.6)	(7.2–12.7)	(8.7–13.4)	(9.4–15.9)	(9.7–17.8)	(10.1–20.7)	(13.2–23.9)	(15.2–25.3)	(13.4–24.1)	(13.6–20.9)
	Dissolved oxygen (%)	115.8	108	98	118.7	105.1	103.5	–	104.8	107.2	113.2	114.9	99	111.2
	Dissolved oxygen (mg L^{-1})	11.5	11.2	10.5	12.9	12.2	11.7	–	11.5	11.3	10.8	10.8	9.3	11.5
	Specific conductivity (µs cm^{-1} @ 25°C)	138	193	166.5	128.7	106.1	134	–	143.8	117	130.7	133.7	166.3	166.5
Mangapiko	Water temperature (°C)	17.8±0.6	15.1±1.6	13.4±1.7	11.1±1.6	10.9±1.6	11.2±1.0	12.6±1.5	13.7±1.4	15.5±1.6	17.7±1.3	19.2±1.9	19.9±1.6	18.1±1.6
		(15.5–19.9)	(10.7–18.8)	(7.2–14.2)	(7.7–16.2)	(7.1–13.3)	(8.3–14.1)	(8.8–16.6)	(9.6–17.3)	(11.2–19.2)	(13.7–20.9)	(15.7–24.9)	(13.8–22.9)	(14.4–21.6)
	Dissolved oxygen (%)	95.6	95.2	95.3	99	101.3	117.4	100	93.4	102.6	112.6	101.8	115.8	99.6
	Dissolved oxygen (mg L^{-1})	9.5	10.1	10.2	10.8	11.3	12.4	10.5	10	9.9	10.8	9.4	10.1	9.9
	Specific conductivity (µs cm^{-1} @ 25°C)	102	122	124.2	122.4	103	124	106	107	107.3	107.1	110.4	93.4	110.8

Table S2. Minimum and median valve lengths of all female and male mussels collected (interquartile ranges in parentheses), sex ratio and total number of mussels sampled at four sites.

	Minimum brooding length (mm) ♀	**Length (mm)** ♀	**Length (mm)** ♂	***N***
E. menziesii				
Ohautira	41	53 (49–58)	54 (50–56)	124
Kahururu	26	47 (36–47)	41 (43–50)	43
Pakoka	23	66 (59–73)	64 (59–72)	43
Mangapiko	36	59 (53–62)	60 (56–62)	50
E. aucklandica				
Ohautira	54	88 (82–90)	86 (82–93)	150
Kahururu	44	71 (62–75)	72 (66–76)	45
Pakoka	49	87 (82–93)	93 (87–97)	51
Mangapiko	40	77 (69–80)	76 (71–79)	47

Table S3. Mann–Whitney *U* test statistics analysing differences in accumulated degree days required to reach brooding onset (Stage 1) and brooding peak (Stage 4) between *Echyridella menziesii* and *E. aucklandica*.

Site	**Mann–Whitney *U***	***P***
Brooding onset		
Ohautira	221	0.018
Kahururu	30	0.01
Pakoka	0	<0.001
Mangapiko	35	<0.001
Brooding peak		
Ohautira	400	<0.001
Kahururu	99	<0.001
Pakoka	448	<0.001
Mangapiko	651	0.002

Table S4. Summary of generalised linear models (beta regression) explaining relationship between accumulated degree days on patterns of brooding proportions of *E. aucklandica* and *E.menziesii* is the beta coefficient (which is the degree of change in the outcome variable for every 1-unit of change in the predictor variable), χ^2 is a partial Wald χ^2 test to assess that the coefficient is significant.

Species and model	β	s.e.	χ^2	*P*	*AME*	s.e.
E. aucklandica						
Onset brooding	0.010	0.004	3.202	**0.006**	0.002	0.0006
Peak brooding	–0.001	0.001	–0.110	**0.002**	–0.0003	0.0001
E. menziesii						
Onset brooding	0.005	0.002	1.979	**0.048**	0.001	0.0005
Peak brooding	–0.001	0.001	–1.875	0.061	–0.0003	0.0001

AME are the average marginal effects (calculation of marginal effects at every observed value of X and averaged across the resulting effect estimates) for each model.

References

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