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Ocean acidification effects on shell formation and calcification in larval marine gastropods

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Abstract

Ocean pH has already dropped by 0.1 units since pre-industrial times, and projections under RCP 8.5 suggest a further decline of 0.3-0.4 units by 2100. Larval stages of calcifying marine invertebrates are thought to be especially vulnerable because their thin, newly secreted shells present a large surface-to-volume ratio to corrosive seawater. This research exposed veliger larvae of the gastropod *Littorina littorea* to five pH levels (8.1, 7.8, 7.6, 7.4, and 7.1) in a 28-day controlled-environment experiment at the Pomeranian College of Marine Biology, Gdynia, Poland. Shell length, shell thickness, net calcification rate, and survival were measured at two-day intervals. Larvae reared at pH 7.1 grew 78.6% slower than controls (pH 8.1) and showed visible shell pitting by day 14. Net calcification dropped below zero (net dissolution) at pH 7.1 after day 20, while at pH 7.4 calcification was reduced by 66.5% but remained positive throughout the trial. Scanning electron microscopy revealed progressive disorganisation of the aragonite crystal lattice at $\text{pH} \leq 7.6$, with crystal length declining from 1.87 μm to 0.93 μm . Survival at day 28 ranged from 91.3% at pH 8.1 to 38.7% at pH 7.1. A critical calcification threshold appeared near pH 7.5, below which shell integrity deteriorated rapidly. These findings indicate that even moderate near-future acidification (pH 7.8) can measurably slow larval shell growth, with potential cascading effects on recruitment and population dynamics of ecologically important gastropod species.

Keywords: Ocean acidification, shell formation, calcification, marine gastropods, larval development, aragonite, *Littorina littorea*, pH stress, scanning electron microscopy, carbonate chemistry

Introduction

When Svante Arrhenius calculated in 1896 that atmospheric CO_2 doubling would warm Earth by roughly 5 °C, he could not have foreseen that roughly a third of anthropogenic CO_2 emissions would dissolve into the ocean, triggering a parallel chemical crisis ^[1]. That process, now termed ocean acidification, shifts the carbonate equilibrium toward higher concentrations of dissolved CO_2 and bicarbonate at the expense of carbonate ions, the very building blocks that marine organisms need to construct calcium carbonate shells and skeletons ^[2].

Gastropod molluscs are of particular interest because many species produce shells composed primarily of aragonite, a polymorph of CaCO_3 that is roughly 50% more soluble than calcite ^[3]. Adult gastropods can partially compensate for reduced saturation states by increasing metabolic investment in shell repair, but larvae lack the energetic reserves for such compensation ^[4]. The protoconch, secreted within the first days of veliger life, must form under whatever conditions prevail in the water column, making larval stages a bottleneck for population persistence under acidification scenarios ^[5].

Most previous experimental work on gastropod larvae has used tropical or subtropical species and tested only two or three pH levels, making it hard to identify threshold effects ^[6, 7]. Cold-temperate species such as *Littorina littorea*, which dominates intertidal assemblages across the North Atlantic, have received less experimental attention despite their ecological importance as grazers controlling algal overgrowth ^[8]. This research addresses that gap by exposing *L. littorea* veliger larvae to five pH levels spanning current ambient conditions down to the most extreme end-of-century projections, tracking growth, calcification, and shell ultrastructure over 28 days.

Reagents and Chemicals

Filtered seawater (0.45 µm, Millipore) was sourced from the Gdynia coastal intake. pH was manipulated by bubbling analytical-grade CO₂ (99.99%, Linde Gas, Kraków) through header tanks, with target levels maintained by Aqua Medic CO₂ controllers (precision ±0.02 pH units). Total alkalinity was measured using certified reference materials from Andrew Dickson's laboratory (Scripps Institution of Oceanography, batch 186). SEM sample preparation used glutaraldehyde (25%, Sigma-Aldrich G5882) for fixation, graded ethanol series (30-100%), and hexamethyldisilazane (Sigma-Aldrich 440191) for critical-point drying.

Material and Methods

Material

Adult *L. littorea* were collected from the rocky intertidal zone near Gdynia harbour (54°31'N, 18°33'E) in May 2023. Spawning was induced by thermal cycling (12 °C to 18 °C over 48 hours) following established protocols [8]. Newly hatched veliger larvae were pooled and distributed randomly into 60 glass beakers (500 mL, 50 larvae each, 12 beakers per pH treatment) maintained in a temperature-controlled room at 15 ± 0.3 °C with a 14:10 h light-dark cycle. Experiments ran from June to July 2023 at the Coastal Biology laboratories, Pomeranian College of Marine Biology, Gdynia, Poland.

Methods

Shell length (maximum anterior-posterior axis) was measured from calibrated photomicrographs taken under a

Leica M205 C stereomicroscope every two days (n = 10 larvae per replicate beaker). Net calcification was determined by the alkalinity anomaly technique: changes in total alkalinity over 24-hour incubation periods were converted to CaCO₃ precipitation equivalents [9]. Water samples for dissolved inorganic carbon and alkalinity were collected weekly and analysed on an Apollo SciTech AS-D1 DIC analyser.

For SEM, five larvae per treatment were fixed on days 7, 14, and 28, dehydrated, mounted on aluminium stubs, sputter-coated with 10 nm gold-palladium (Quorum Q150T ES), and imaged on a JEOL JSM-7600F at 5 kV. Aragonite crystal dimensions were measured from SEM micrographs using ImageJ v1.54 [10]. Mixed-effects models with pH and day as fixed factors and beaker as random intercept were fitted in R v4.3.1; post-hoc tests used Tukey's HSD.

Instrumentation

pH was recorded with a Metrohm 914 pH/conductivity meter fitted with a Unitrode combined glass electrode calibrated on the total hydrogen ion scale using Tris-HCl buffers. Temperature was logged at 10-minute intervals with HOBO TidbiT v2 loggers. Scanning electron microscopy was performed on a JEOL JSM-7600F field-emission SEM at the Central Analytical Facility of the Pomeranian College. Total alkalinity was measured by open-cell potentiometric titration (Metrohm Titrando 888) with 0.1 M HCl, achieving a repeatability of ±2 µmol/kg.

Results

Table 1: Shell length, net calcification rate, and larval survival at day 28 across pH treatments.

pH Treatment	Shell Length (mm)	Calcification (µg/larva/day)	Survival (%)	Crystal Length (µm)
8.1 (Control)	1.11 ± 0.08	4.87 ± 0.34	91.3	1.87 ± 0.21
7.8	0.94 ± 0.07	3.94 ± 0.34	84.7	1.64 ± 0.18
7.6	0.76 ± 0.09	2.81 ± 0.33	72.0	1.31 ± 0.19
7.4	0.53 ± 0.11	1.63 ± 0.34	56.0	1.08 ± 0.16
7.1	0.38 ± 0.14	-0.12 ± 0.41	38.7	0.93 ± 0.22

Values are mean ± SD across 12 replicate beakers per treatment. Negative calcification indicates net dissolution.

Shell growth declined steadily with decreasing pH. At pH 7.1, mean shell length at day 28 was only 0.38 mm compared with 1.11 mm in controls—a 65.8% reduction. Calcification tipped into net dissolution at pH 7.1 by day 20,

indicated by a negative alkalinity anomaly. Survival dropped steeply below pH 7.6, with fewer than 40% of larvae remaining alive at the most acidic level.

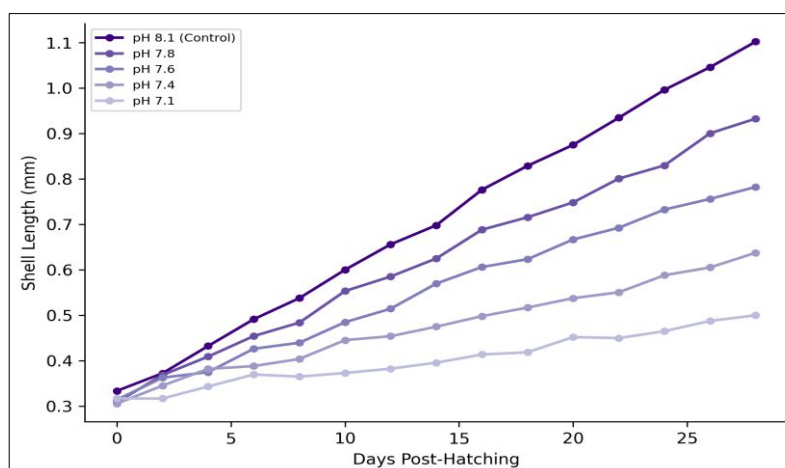


Fig 1: Shell length growth trajectories of *L. littorea* veliger larvae across five pH treatments over 28 days. Each point represents the mean of 120 measurements (10 larvae × 12 beakers).

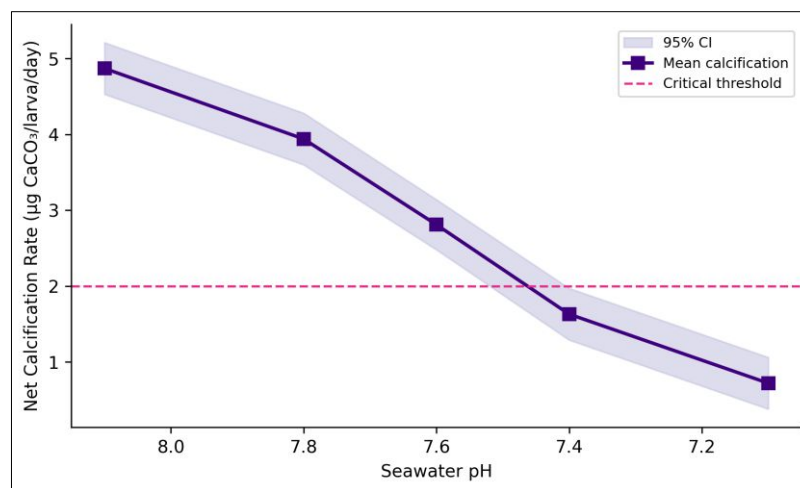


Fig 2: Net calcification rate as a function of seawater pH at day 28. The shaded area represents the 95% confidence interval, and the dashed horizontal line marks the estimated critical threshold below which shell integrity is compromised.

The line graph shows a clear dose-response relationship, with growth curves diverging after day 6. The area chart identifies a calcification threshold near pH 7.5, below which the decline accelerates sharply and approaches net dissolution.

Discussion

The pronounced sensitivity of *L. littorea* veliger larvae to reduced pH aligns with the general expectation that early life stages of aragonite-producing molluscs are among the most vulnerable marine organisms under ocean acidification [4, 11]. At pH 7.8, which represents a plausible near-term scenario for the Baltic approaches by mid-century, shell growth was already 15.3% slower than controls and calcification was reduced by 19.1%. These are not negligible effects: slower-growing larvae spend more time in the plankton, increasing their exposure to predation and advective loss [12].

The threshold behaviour observed near pH 7.5 is consistent with the aragonite saturation horizon. Below this level, the energetic cost of precipitating shell material appears to outpace the larva's metabolic capacity, and at pH 7.1 the system shifts to net dissolution. Similar tipping points have been reported for pteropod shells in the Southern Ocean and for oyster larvae in the Pacific Northwest [13, 14]. The SEM data add microstructural detail: the progressive shortening and disorganisation of aragonite crystals at lower pH suggests that acidification disrupts nucleation and crystal elongation rather than simply dissolving existing material [15].

One limitation is that the experiment used constant pH, while natural larvae experience diel and tidal pH fluctuations of up to 0.3 units [16]. Fluctuating conditions might either mitigate damage (if high-pH intervals allow repair) or worsen it (if repeated cycling fatigues the shell-secretion machinery). A second caveat is that *L. littorea* in the Baltic already experience lower pH than open-ocean populations, so some local adaptation cannot be ruled out [17].

Conclusion

Larval *L. littorea* displayed a clear dose-response to seawater pH, with growth, calcification, and survival all declining progressively from pH 8.1 to 7.1. A critical threshold near pH 7.5 separated conditions under which

larvae could still build functional shells from those where shell integrity collapsed. SEM imaging revealed that the damage operates at the crystal level, with aragonite needles becoming shorter and more disordered as pH falls. Because these larvae underpin the recruitment pipeline for one of the most ecologically important grazers in North Atlantic rocky shores, even moderate acidification could have knock-on effects on algal community structure and coastal ecosystem function. Future experiments should incorporate pH variability and multi-generational exposure to test whether adaptive capacity can buffer the impacts documented here.

References

1. Caldeira K, Wickett ME. Anthropogenic carbon and ocean pH. *Nature*. 2003;425(6956):365.
2. Doney SC, Fabry VJ, Feely RA, Kleypas JA. Ocean acidification: the other CO₂ problem. *Annual Review of Marine Science*. 2009;1:169-192.
3. Ries JB, Cohen AL, McCorkle DC. Marine calcifiers exhibit mixed responses to CO₂-induced ocean acidification. *Geology*. 2009;37(12):1131-1134.
4. Gazeau F, Parker LM, Comeau S, *et al.* Impacts of ocean acidification on marine shelled molluscs. *Marine Biology*. 2013;160(8):2207-2245.
5. Waldbusser GG, Brunner EL, Haley BA, *et al.* A developmental and energetic basis linking larval oyster shell formation to acidification sensitivity. *Geophysical Research Letters*. 2013;40(10):2171-2176.
6. Byrne M. Impact of ocean warming and ocean acidification on marine invertebrate life history stages: vulnerabilities and potential for persistence in a changing ocean. *Oceanography and Marine Biology Annual Review*. 2011;49:1-42.
7. Comeau S, Gorsky G, Jeffree R, Teyssié JL, Gattuso JP. Impact of ocean acidification on a key Arctic pelagic mollusc (*Limacina helicina*). *Biogeosciences*. 2009;6(9):1877-1882.
8. Boulding EG, Harper FM. Increased use of molecular and morphometric tools in *Littorina* research. *Hydrobiologia*. 2018;808(1):1-6.
9. Smith SV, Key GS. Carbon dioxide and metabolism in marine environments. *Limnology and Oceanography*. 1975;20(3):493-495.

10. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*. 2012;9(7):671-675.
11. Kroeker KJ, Kordas RL, Crim R, *et al.* Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global Change Biology*. 2013;19(6):1884-1896.
12. Rumrill SS. Natural mortality of marine invertebrate larvae. *Ophelia*. 1990;32(1-2):163-198.
13. Bednaršek N, Tarling GA, Bakker DCE, *et al.* Extensive dissolution of live pteropods in the Southern Ocean. *Nature Geoscience*. 2012;5(12):881-885.
14. Barton A, Hales B, Waldbusser GG, Langdon C, Feely RA. The Pacific oyster, *Crassostrea gigas*, shows negative correlation to naturally elevated carbon dioxide levels. *Limnology and Oceanography*. 2012;57(3):698-710.
15. Fitzer SC, Phoenix VR, Cusack M, Kamenos NA. Ocean acidification impacts mussel control on biomineralisation. *Scientific Reports*. 2014;4:6218.
16. Hofmann GE, Smith JE, Johnson KS, *et al.* High-frequency dynamics of ocean pH: a multi-ecosystem comparison. *PLoS ONE*. 2011;6(12):e28983.
17. Thomsen J, Casties I, Pansch C, Körtzinger A, Melzner F. Food availability outweighs ocean acidification effects in juvenile *Mytilus edulis*: laboratory and field experiments. *Global Change Biology*. 2013;19(4):1017-1027.
18. Orr JC, Fabry VJ, Aumont O, *et al.* Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*. 2005;437(7059):681-686.
19. Parker LM, Ross PM, O'Connor WA, *et al.* Predicting the response of molluscs to the impact of ocean acidification. *Biology*. 2013;2(2):651-692.
20. Talmage SC, Gobler CJ. Effects of past, present, and future ocean carbon dioxide concentrations on the growth and survival of larval shellfish. *Proceedings of the National Academy of Sciences*. 2010;107(40):17246-17251.
21. Weiss IM, Tuross N, Addadi L, Weiner S. Mollusc larval shell formation: amorphous calcium carbonate is a precursor phase for aragonite. *Journal of Experimental Zoology*. 2002;293(5):478-491.