Hang on Tight: Association with Algae During Egg Development Limits Invasiveness in *Haloa japonica*

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Abstract

Characterizing population demography and habitat selection is one of the key factors in predicting invasion outcomes. Native cephalaspidean *Haminoea vesicula* and introduced *Haloa japonica* on San Juan Island, WA are distinct in their egg-laying substratum preference. The former is indiscriminate in substratum use whereas the latter almost exclusively oviposits on macrophytes and algae. We investigated whether occurrence of macroalgae or macrophytes is essential to the development of cephalaspidean eggs. We reciprocally transplanted native

Haminoea vesicula and introduced *Haloa japonica* from their respective habitats in False Bay and Argyle Lagoon on San Juan Island, WA, and replicated reciprocal rearing in the lab. Our field surveys and transplant experiments indicate that oviposition on filamentous algae leads to higher rates of egg development in *H. japonica*. This strong algal association could constrain adult habitat selection to sites with high macroalgal/macrophyte availability, controlling the invasive potential of *H. japonica.* Such sites may not be optimal for *H. vesicula* for oviposition, even in the absence of *H. japonica*, as *H. vesicula* egg masses ruptured pre-hatching when reared in reciprocal conditions in the lab. Our field survey also revealed a third species of cephalaspidean occurring in these well-studied nature preserves—*Haminoea virescens*—and enabled comparative analyses of their egg mass biology.

Introduction

Elucidating the ecophysiological and demographic determinants of community assembly is key to understanding the maintenance of diversity, and to predicting invasive potential, coexistence and persistence of functionally similar native–invader pairs (Violle et al., 2012). Ontogenetic niche shifts can have significant implications for species interaction and coexistence by limiting similarity (Werner & Gilliam, 1984). For example, hypoxic stress due to egg mass size, salinity and temperature fluctuations constrains maximum egg mass size and developmental schedules (Chaffe *&* Strathmann, 1984). Eggs at the center of egg clutches often experience oxygen levels approaching their tolerance limits in natural habitats (Cohen *&* Strathmann, 1996). Some sympatric species of intertidal opisthobranchs are partitioned into distinct habitats, due to species-specific associations with photosynthetic organisms that alleviate periodic hypoxic stress in egg masses (Fernandes & Podolsky, 2011; Woods *&* Podolsky, 2007). Specific oviposition microsite requirements may be quite different from optimal adult habitat, constituting an ontogenetic tradeoff (von Dassow & Strathmann, 2005). We ask whether early development niche differentiation in native *Haminoea vesicula* (Gould, 1855) and introduced *Haloa japonica* (Pilsbry, 1895 syn. *Haminoea japonica*) confers competitive coexistence or invasion resistance. The notion that larval characteristics strongly influence dispersal and adult distribution is nearly paradigmatic (Barosso et al., 2022, Jablonski & Lutz, 1983). Our study sought to test if habitat filtration in early development, *i.e.* egg mass survival, is a key factor determining resultant adult habitat selection that dominates over filtration at later life history stages at potential recruitment sites.

Haminoea vesicula is native to the Salish Islands and produces type A egg masses (Strathmann, 1987) in thick ribbons approximately 5 mm wide, coiled on eelgrass, algae, shells and rocks. Capsules are aligned in a single row or closely packed up to four or five across in a string that is arranged in a flattened spiral (Strathmann, 1987). Christened with synonymous nomina *Haminoea callidegenita* by Gibson and Chia (1989) when it was first discovered from Lopez Island by Dr. R. L. Fernald from the University of Washington, *Haloa japonica* (genus designation sensu Oskars & Malaquias, 2019) is an introduced species in the Salish Sea native to the West Pacific (Gosliner & Behrens, 2006). It produces thick type A egg masses in a string of about 100 well-separated capsule spirals, with each capsule containing one egg. Our personal observations suggest that both species are sympatric *sensu lato*, as adults show strong habitat partitioning concordant with their egg-laying sites. Eggs of *H. vesicula* show less specific substrate association in the intertidal (False Bay), whereas *H. japonica* is almost exclusively associated with algae in warmer subtidal lagoon environments (e.g. Argyle Lagoon).

Historical records of *H. japonica* in False Bay where eelgrass beds were once more common (Gibson, 1993) further affirm our suspicions that *H. japonica* shows strong associations with macro photosynthesizers, and their abundance and distribution may track that of macrophytes and macroalgae. Preferential oviposition and larval settlement on filamentous algae is a known life history strategy of *H. japonica*, where algal association cues more eggs to hatch as crawl-away juveniles in this poecilogonous species (Allen, 2014). Filamentous algae While *H. vesicula* is a generalist in oviposition substratum preference, it is also known locally to suffer from the paucity of suitable egg-laying sites, thus may be more predisposed to displacement by an invader competing for oviposition sites. Enamored by these observations, we pursued the question of whether *H. vesicula* and *H. japonica* are divergent in habitat use due to limits in environmental tolerance as curtailed by symbiotic associations at early life stages. We examined embryonic development in egg masses through the veliger stage for these two haminoeids in reciprocal conditions representative of the two habitats to determine whether these species present with different levels of mortality, rate of post-stress recovery, and developmental schedules. To reproduce these conditions, we mimicked the temperature and biotic conditions that are present in the respective habitats of the species in a laboratory setting. We hypothesized that *H. japonica* and *H. vesicula* would exhibit higher developmental delays when reciprocally transplanted to heterospecific habitat.

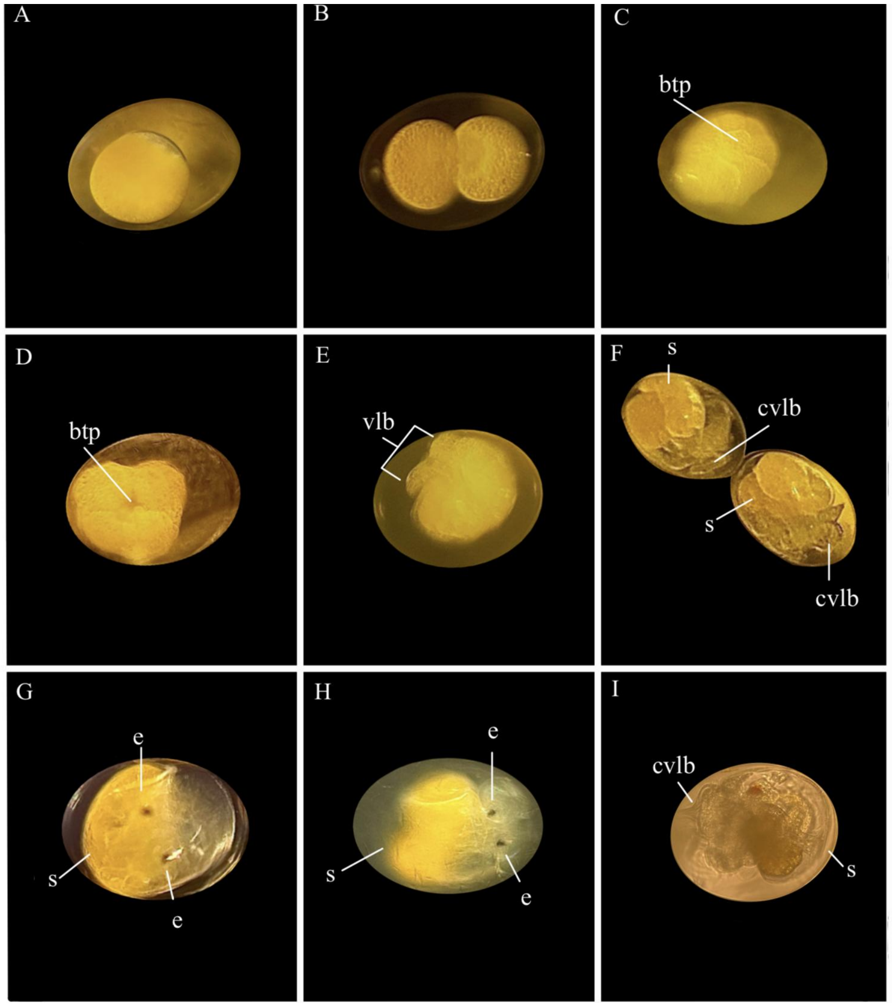
Materials and Methods

Collection and Evaluation of Study Organisms

Twenty-eight egg clutches of *H. vesicula* and *H. japonica* were collected from both False Bay and Argyle Lagoon, for a total of 56 egg clutches (Fig. 1). We trimmed each egg clutch to equal size to minimize differential development rate due to oxygen exposure (*vid.* Chaffe & Strathmann, 1987). Egg masses were scored under 40× bright-field compound microscopes and assigned the following developmental stages: fertilized-to-gastrula (1), pre-veliger (2), veliger (3), and hatched (4) (Fig. 2). As embryos were largely synchronous in development within each egg mass, they were scored according to the developmental stage that the majority of embryos were in.

Fig. 1. Map of field transplant site at Argyle Nature Preserve annotated with the placement of four thermologgers and field transplants. Inset map shows *H. vesivula* collection locality at False Bay Nature Preserve and the extent of current map for Argyle Nature Preserve on San Juan Island.

Fig. 2. Developmental stages of *H japonica* (A–H) and *H. vesicula* embryo (I). A) Zygote, B) 2-cell stage, C) early formatting of the blastopore, D) gastrulation with visible blastopore, E) pre-veliger with early formation of the velar lobes, F) veliger with visible ciliated velar lobes, G) advanced embryo with no ciliated velar lobes, H) early juvenile, I) *H. vesicula* veliger. Abbreviations: (btp) blastopore, (vlb) velar lobes, (s) shell, (cvlb) ciliated velar lobes, (e) eyes.



All clutches of both species had reached at least the gastrula stage by the time we collected them. Stage 1 (gastrula) was characterized by the presence of the blastopore but a lack of larval structures such as a velar lobes and a shell. We did not observe movement at this stage. Stage 2 (early veliger) consisted of the emergence of structures such as the velar lobes and cilia, allowing for slow rotation of the embryo. At this stage, the formation of the germ layers occurs and early larval structures can be observed. At stage 3 (veliger), the larval shell, ciliated velum, and steady rotation within the capsule are observable. We did not always observe movement by the veliger at this stage, but defined structures and ciliary movement were always present. We categorized hatched, free-swimming veligers as stage 4. The egg masses themselves at this stage were deteriorating and often covered in diatoms. Collected egg masses were at different stages. To quantify development, rate of development was calculated by the difference of the first and the last scoring of the egg masses, i.e. the difference between the first and the last stage the embryos were in.

Environmental Data Tracking

We deployed HOBO pendant thermologgers at Argyle Lagoon at four sites with evident oviposition: 1) on algal mats in the lagoon, 2) on mud in the lagoon, 3) ~0.5 m deep in the ocean channel, and 4) ~1 m deep in the ocean channel, to determine local temperature and light intensity variation at 30-min intervals. To determine parameters for our common garden experiments, we measured water temperature and dissolved oxygen at both sites on a single low tide (-1.0 m) with a YSI ProODO Optical Dissolved Oxygen probe at both field sites during egg mass collections. We deployed the loggers at Argyle Lagoon two days prior to deploying our field treatments. We collected the loggers, along with our field treatments, after five days.

Common Garden Experiment

We manipulated conditions in six 1.5 m x 0.9 m flow-through seawater tanks to feature key characteristics of the natural habitats. We filled the three tanks slated for “lagoon” treatment to mimic *H. japonica* habitat with filamentous algae from Argyle Lagoon and provided them with a low water flow (0.42 L min^{-1}) such that the tank warmed during the daytime (11–32°C). We did not add algae to the three tanks destined for “ocean” treatment to mimic *H. vesicula* habitat, but provided them with a steady water flow (1.71 L min^{-1}) such that the tank was kept at field water temperature (11–23°C). We deployed Onset HOBO TidbiT 400ft thermologgers in each tank to record temperature and light.

18 egg masses from each species were placed in the tanks, nine in their natural and nine in their reciprocal conditions. In each tank, six egg masses were placed at random positions, summing up to 6 tanks containing 36 egg masses in total. Each tank received six egg masses positioned at random (n = 36), totalling 18 from both species, nine of which were in reciprocal conditions and nine in their natural conditions. Fences made of plankton net were arranged to partition each tank into six sections, to prevent intermixing of the egg masses while allowing seawater flow. Egg masses were scored three times over the course of 6 days: 1 day before treatment exposure, and 2 and 4 days after the start of treatment exposure.

Field Reciprocal Transplant

To corroborate our observations in lab experiments, we conducted reciprocal experiments *in situ* by placing egg masses of both species at reciprocal sites: Argyle Lagoon (48°31'13.6"N 123°00'46.5"W) and Argyle Ocean (48°31'13.7"N 123°00'51.8"W). 10 egg masses of *H. vesicula* from False Bay (48°29'23.6"N 123°04'02.2"W) were collected and five were placed at their natural site (ocean) and five at their reciprocal site (lagoon). Five *H. japonica* egg masses were placed at their natural site (lagoon) and five at their reciprocal site (ocean). We placed egg masses that had been scored and were ready to be outplanted to the field into plastic storage containers with mesh sides to allow seawater exchange. We tied the containers into a bundle and secured them to our thermologger setups. We placed a total of 10 egg masses (n = 5 of each species) at each field site. We attached a small (d = 3 cm) rock to each container at the Argyle Ocean site to keep experimental sets on the benthos. The containers remained in the field for 3 d (field setup see Figure 3).

Figure 2: Field deployment setup, plastic containers each contain one egg mass. Buoy was hold in place with a brick.

Data Analysis

We conducted statistical analysis in *R* version 4.0.3 (R Core Team, 2020). Shapiro-Wilk tests were performed to test for normality of data and Fligner-Kileen tests for homogeneity of variance. For *H. japonica* in the field, a t-test was carried out for pairwise comparison between treatments (lagoon vs. ocean) . For the same species in the laboratory, we conducted a zero-inflated Poisson regression to test for differences between the ocean and lagoon treatment.

No statistical tests were carried out for *H. vesicula* development rates, as those could not be used for further analysis.

Results

Environmental Conditions at Field Sites and in Laboratory

Reported temperatures and light intensities are means and standard deviation. At the field sites, temperature was on average higher in the ocean than in the lagoon (see Table 1). Light intensity was on average higher in the lagoon than in the ocean (see Table 1). In the laboratory, temperature was on average higher in the lagoon treatment than in the ocean treatment (see Table 1). Light intensity was on average slightly higher in the lagoon treatment than in the ocean treatment (see Table 1). Temperature peaks coincide with light intensity peaks representing day/night cycles (see Figure 4 and 5). Laboratory temperature could be maintained similar to the field conditions (see Figure 4 and 5).

Table 1. Logged temperature (°C) and light intensity (lux) from laboratory treatments and field sites. Temperatures and light intensities were recorded in 30-min intervals over a period of 5 days

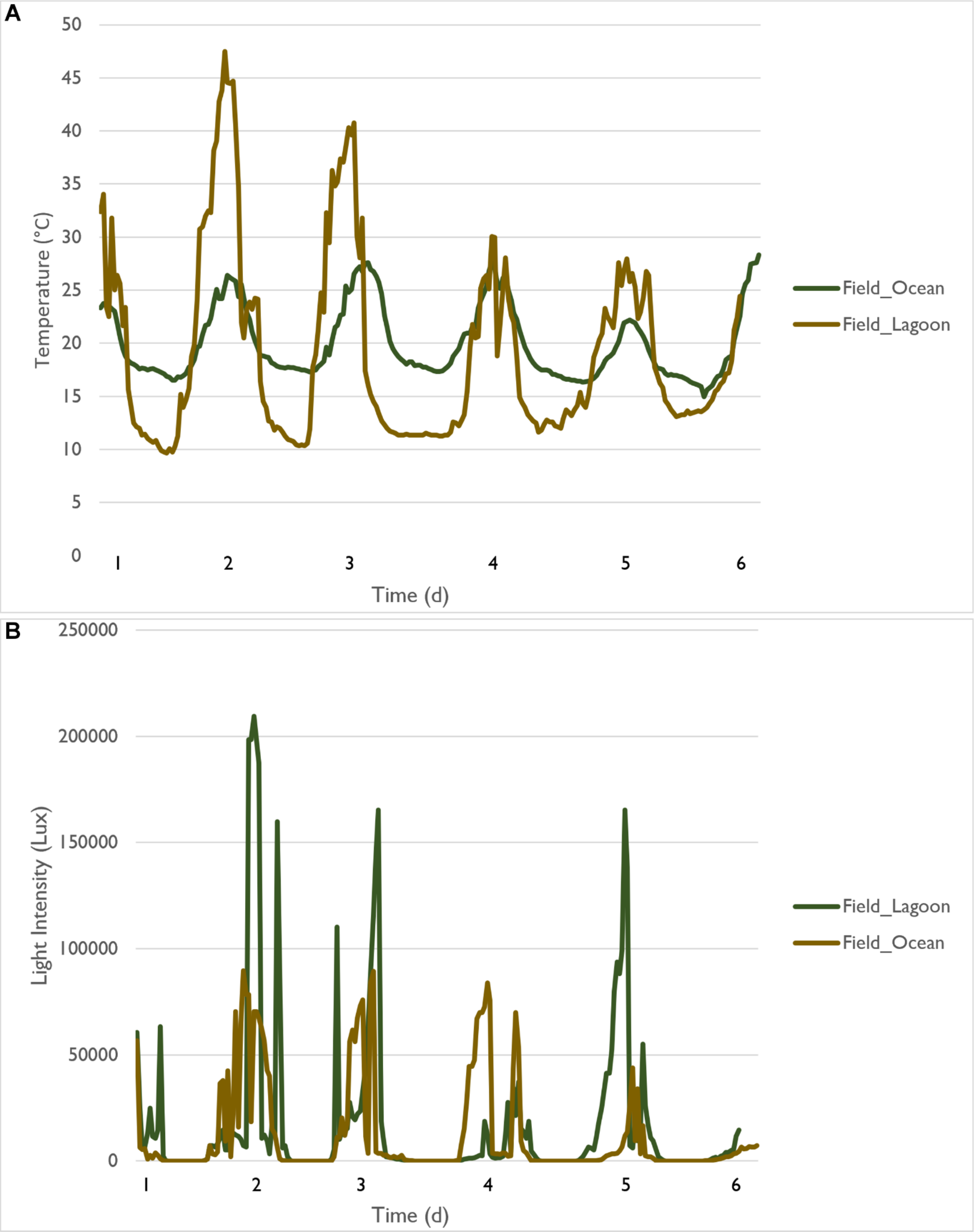
(03.07.23-08.07.23). Reported are means, standard deviation, maximum and minimum temperature/light intensity. SD= standard deviation

Treatment	**Mean + SD Temperature (°C)**	**Mean + SD light intensity (lux)**
Field (Lagoon)	19.06 ± 8.77	17537.86 ± 39373.17
Field (Ocean)	20.02 ± 3.49	12099.32 ± 22860.62
Laboratory (Lagoon)	18.38 ± 7.35	25164.87 ± 32790.02
Laboratory (Ocean)	14.94 ± 3.84	26319.54 ± 49039.70

Treatment	**Maximum Temperature (°C)**	**Minimum Temperature (°C)**	**Maximum light intensity (lux)**	**Minimum light intensity (lux)**
Field (Lagoon)	47.50	9.67	209423.6	0
Field (Ocean)	28.34	14.95	89556.15	0
Laboratory (Lagoon)	32.39	11.09	187379	0
Laboratory (Ocean)	23.31	11.25	95067.3	0

Table 2. Temperature (°C) and dissolved oxygen (mg L^{-1}) measurements take YSI ProODO Optical Dissolved Oxygen Meter probe during snail and egg mass collection.

Sites	**Depth (m)**	**Temperature (°C)**	**Dissolved oxygen (mg L^{-1})**
False Bay	0.15	24.8	10.8
Argyle Lagoon	0.30	20.8	15.8
Argyle Lagoon	0.75	20.3	13.2
Argyle Ocean	0.30	22.0	14.6
Argyle Ocean	0.75	20.1	14.7


A
Temperature (°C)
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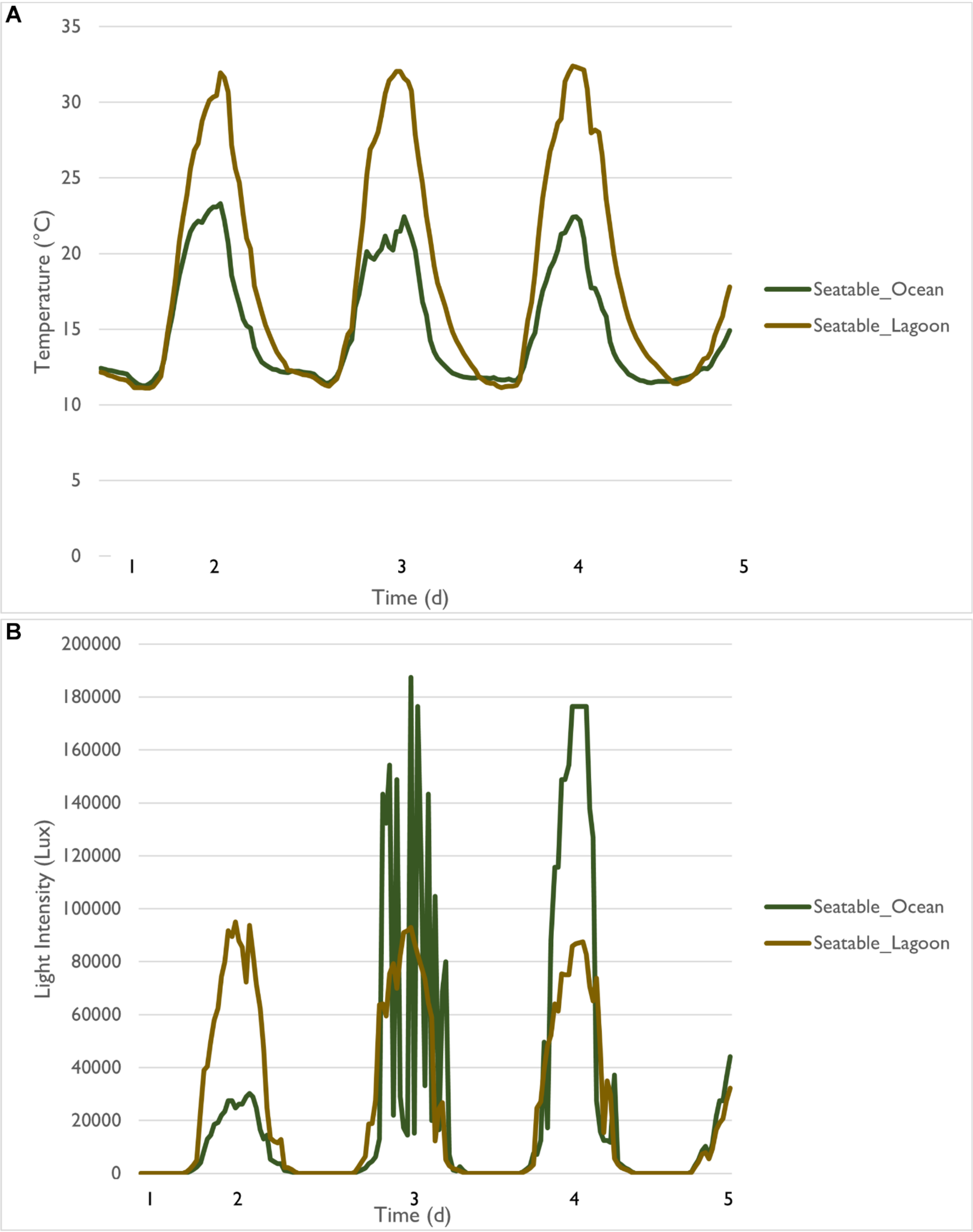
45
40
35
30
25
20
15
10
5
0
1
2
3
4
5
6
Time (d)
Field_Ocean
Field_Lagoon
B
Light Intensity (Lux)
250000
200000
150000
100000
50000
0
1
2
3
4
5
6
Time (d)
Field_Lagoon
Field_Ocean

Figure 4. Logged temperature (°C; A) and light intensity (lux; B) at the field site (Argyle Lagoon/Ocean). Days 1–6 represent 3–8 July 2023. Maximum/minimum peaks in temperature and light intensity show day/night cycles. Temperature peaks coincide with light intensity peaks.


A
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Temperature (°C)
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Time (d)
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80000
60000
40000
20000
0
Light Intensity (Lux)
1
2
3
4
5
Time (d)
Seatable_Ocean
Seatable_Lagoon

Figure 5. Logged temperature (°C; A) and light intensity (lux; B) in the laboratory (Lagoon/Ocean). Days 1–5 represent 3–7 July 2023. Maximum/minimum Peaks in temperature and light intensity show day/night cycles. Temperature peaks coincide with light intensity peaks.

Development of Egg Masses in Reciprocal Field Transplants

Our deep-ocean buoy and data logger were lost during the field deployment period. The shallow-ocean buoy and attached treatments were found by a member of the public who contacted us and left the buoy on dry land in full sun on the last day of field experiments. As such almost all egg clutches in this experimental set completely desiccated in the sun, shrinking our intended sample size drastically.

The rate of development represents the difference between the first scoring of developmental stages and the last scoring and is quantified by a number of stages the embryo in the egg developed. Only *H. japonica* development was assessed, as *H. vesicula* egg masses disintegrated and could not be used for further analysis.

H. japonica developed faster in its reciprocal habitat, with rates of 1.25 ± 0.83 stages vs. 0.67 ± 0.47 stages in the original habitat (see Figure 6) .

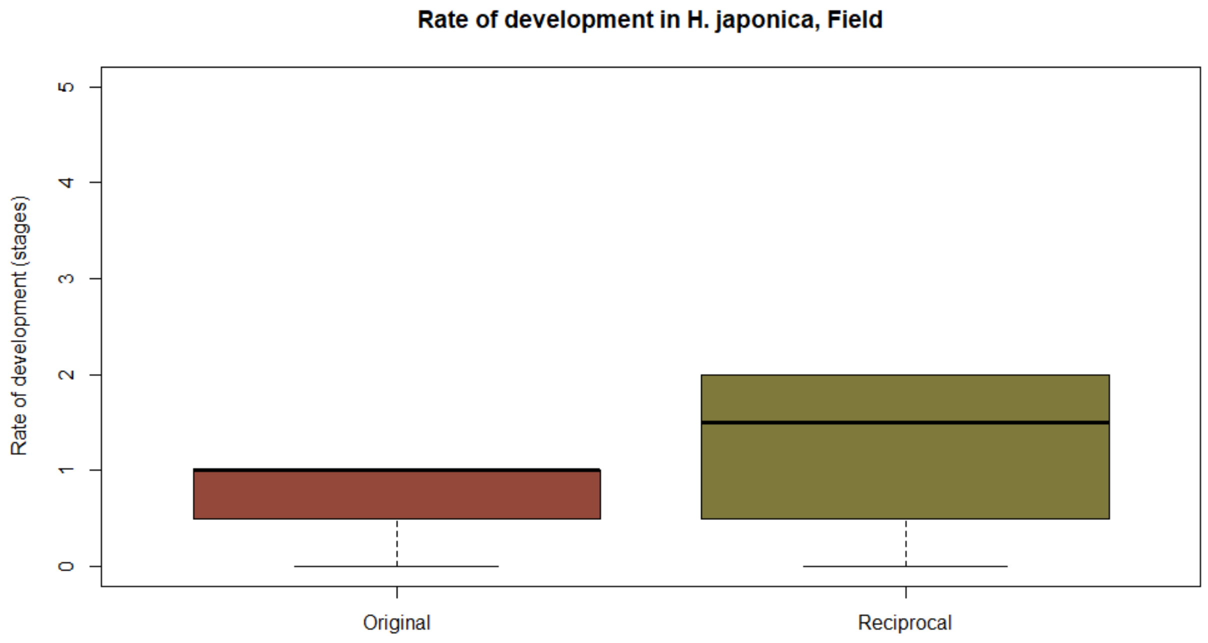

Figure 6. Rate of development of egg cases for *H. japonica* in field transplants, original (n = 3) and reciprocal (n= 4) sites. Original site for the species is Argyle Lagoon whereas the reciprocal site is Argyle Ocean. Rate of development is computed as the number of stages advanced by eggs throughout the experiment.

Development of Eggs in Reciprocal Seatable Transplants

Most *H. vesicula* egg masses disintegrated and could not be scored for further analysis. The developmental rate was 0.33 ± 0.67 stages in the original and 0.71 ± 0.70 stages in the reciprocal conditions (see Figure 7).

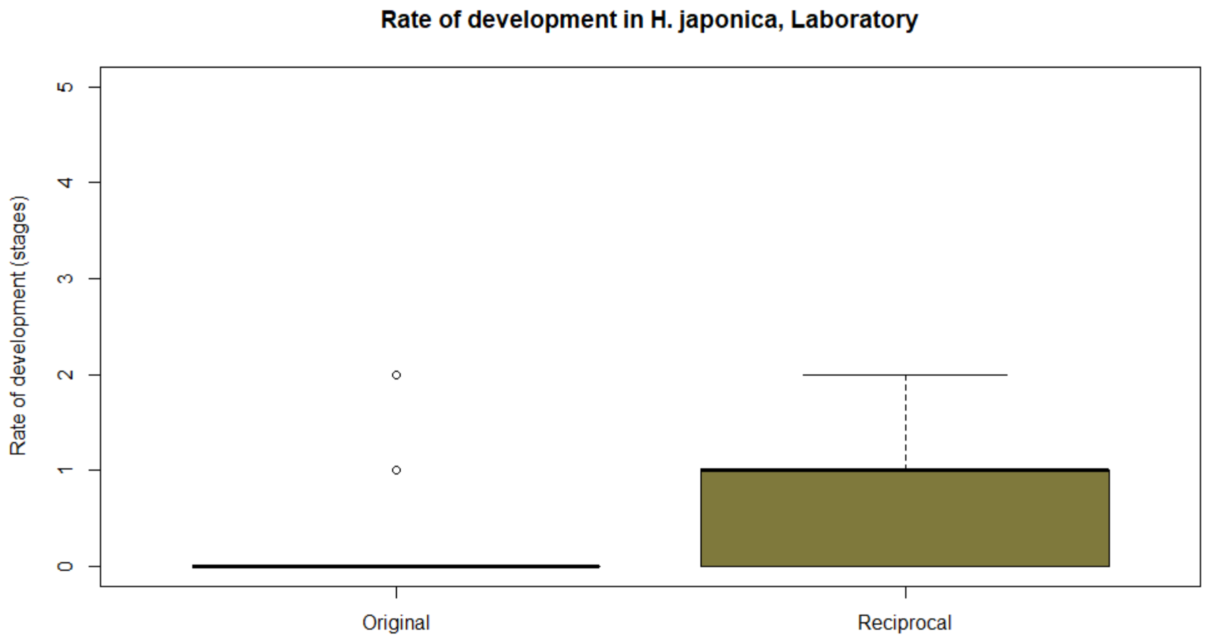

Figure 7. Rate of development of egg cases for *H. japonica* in laboratory transplants, original (n = 9) and reciprocal (n= 7) sites. Original site for the species is Argyle Lagoon whereas the reciprocal site is Argyle Ocean. Rate of development is computed as the number of stages advanced by eggs throughout the experiment.

Statistical Analysis

For H. japonica, the rates of development were not significantly different between the lagoon and ocean treatment (t-test, t= -0.9, p= 0.49). For the laboratory treatment, a zero-inflated Poisson regression was used to test for differences between the ocean and lagoon treatment. Differences were not significantly different (Estimate= -0.2, Standard Error= 1.04, z value= -0.19, p= 0.85).

Notes on Haminoea *spp. Assemblage in Argyle Lagoon and False Bay*

Four individuals resembling *H. japonica* collected from Argyle Lagoon deposited type A egg masses in the lab that superficially resembled those of *H. vesicula.* This mismatch was clarified by descriptions in Richards (1922, 1923) of oviposition and early development of native cephalaspideans, identifying our specimens as *Haminoea virescens*. Unlike Richards's documentation and sketches portraying one-embryo-per-capsule, we observed multiple embryos cohabiting one egg capsule in many egg masses examined (Fig. 8). We think it noteworthy to report the co-occurrence of *H. virescens* at Argyle Lagoon with *H. japonica,* such that future researchers are aware of this assemblage, and that the question of whether species assemblage in the lagoon shows significant short term turnovers may be investigated. .

Despite our extensive survey efforts, after examining over 100 individuals, we did not recover a single individual of *H. vesicula* at Argyle Lagoon where they were documented to occur by von Dassow and Strathmann (2005). However, *H. japonica* was abundant amongst filamentous algae, attaining densities exceeding 2 individuals per squared centimeter in certain areas. We provide below a comparative table of traits to distinguish the egg masses of these three species (Table 3). *H. japonica* produces starkly distinct egg masses. We propose distance of egg columns in the egg mass, size of egg mass and egg-laying habits as diagnostic features between *H. vesicula* and *H. virescens* **(Fig. 9, Fig. 10)**.

Table 3. Species comparison among egg mass deposition and structure.

Traits	***H. japonica***	***H. vesicula***	***H. virescens***
Egg Diameter	00-240 µm	0 µm	~ 81.3 µm

Egg Mass Type	ype A	ype A	Type A
Egg Colour	ellow	ellow	Pale Yellow
Arrangement in Capsule	ne egg per capsule	ne egg per capsule	Frequent multiput embryos per capsule
Oviposition Habit	lgae	gae, shells, or stones	algae
Poecilogony	es	o	No
Density in Capsule	ell separated	losely-packed	Egg string packed
Egg Clutch Size	0.5 cm x 1.5 cm	5 mm	W 10 mm

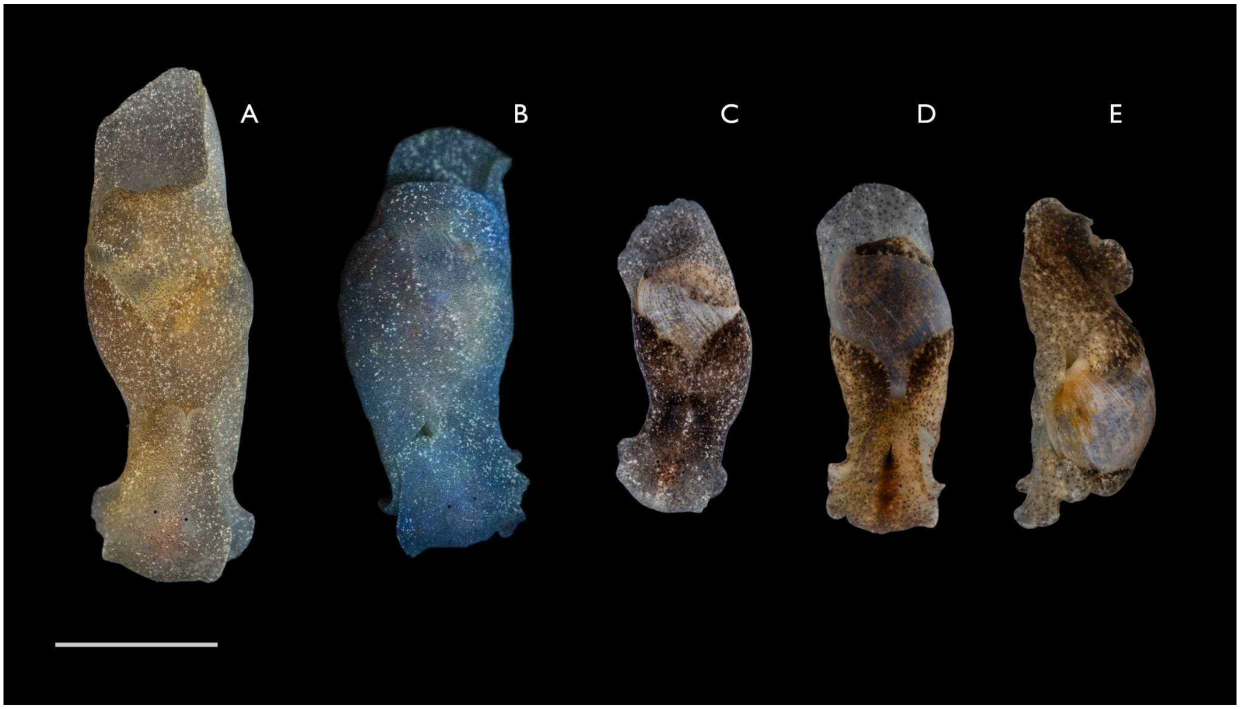

Figure 8. Photos of live *Haminoea* spp. A. *H. virescens* dorsal view. B. *H. virescens* biofluorescing under 365-nm ultraviolet exposure. Biofluorescence was not observed for other species. C. *H.*

vesicula dorsal view D. *H. japonica* dorsal view. E. *H. japonica* lateral view. Scale bar represents 1 cm.

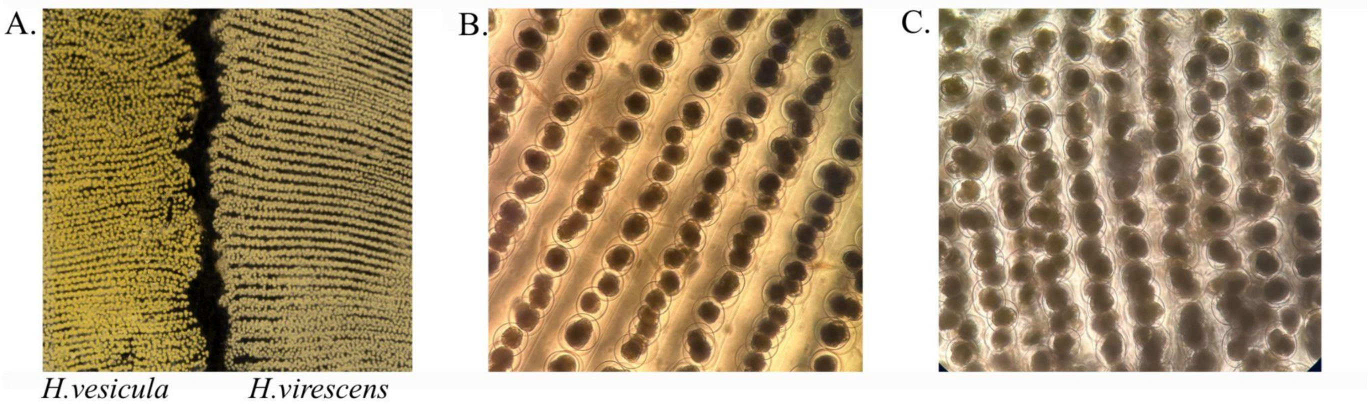

Figure 9. A) Side-by-side comparison of egg mass patterns from *H. vesicula* (right) and *H. virescens* (left). B) *H. virescens* aligned egg capsules. C) *H. vesicula* closely-packed egg capsules.

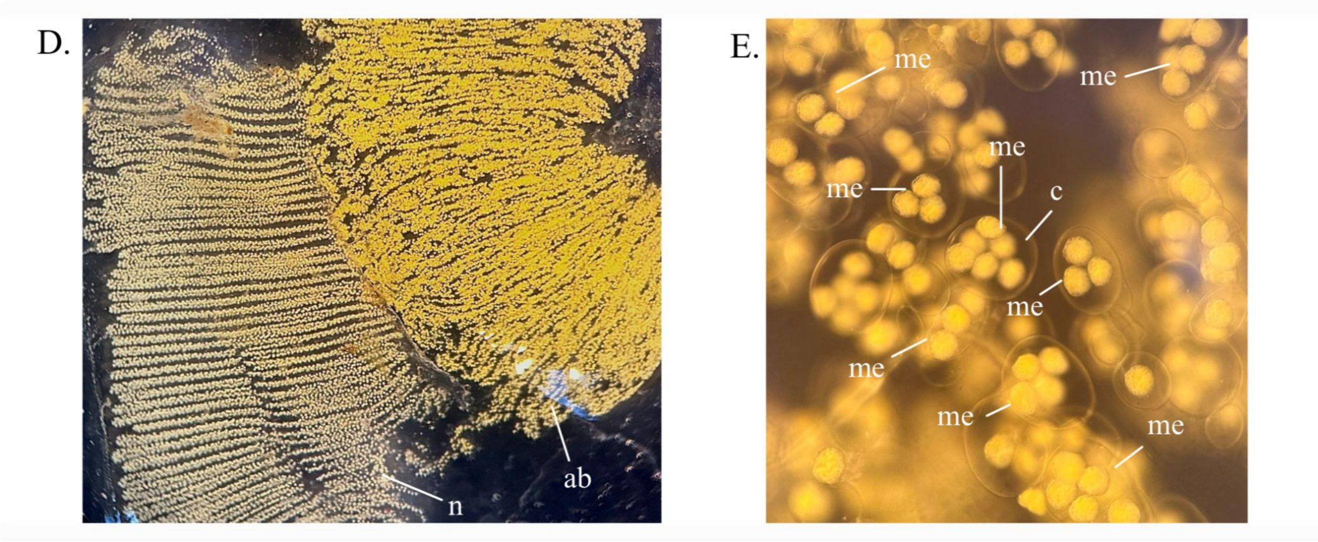

Figure 10. D) Right side egg mass with normally aligned egg capsules and abnormal left side egg mass with distorted egg capsules. E) Multiple embryos per capsule. Abbreviations: (c) egg capsules, (me) multiple embryos, (n) normal, (ab) abnormal.

Discussion

Seatable Environmental Conditions Broadly Match Field Conditions

We found temperature and light to be similar in lab and field sites. We found some differences in egg development, which we suggest derive from causes other than light and temperature. Comparing the field and laboratory conditions, the lagoon showed higher temperature peaks and fluctuations than the ocean. The shallow lagoon at Argyle is periodically isolated from the ocean, during which it experiences rapid warming. In contrast, the ocean channel is well-circulated and therefore buffered from temperature fluctuations. Light intensity was higher in the lagoon in the field, contrary to laboratory conditions. Experimental limitations such as buoys being washed ashore and directional light recorded by light sensors located on one side of the loggers, algae cover and changes in depth due to changing tides may explain this discrepancy.

Developmental performance of H. japonica *is contingent on algal association*

Despite significant losses in sample size, field transplants suggested habitat specificity in *H. japonica*. Egg masses developed more rapidly in Argyle Lagoon (i.e., the original site for the species) in the vicinity of algae and barely progressed in development in the absence of algae (Fig. 6). We speculate that hypoxic stress in the absence of algal symbionts caused delays in the development of *H. japonica* egg masses (Phillips & Moran, 2015). Compared to *H. vesicula*, *H. japonica* traversed more developmental stages in Argyle Lagoon. Due to loss of data for *H.*

vesicula for comparison, we consider this as a preliminary indication that *H. japonica* egg masses outperform *H. vesicula* when deposited on algae.

We cannot establish whether *H. vesicula* shows reduced performance in the vicinity of algae in our field experiments, though it is clear that the obligate association of *H. japonica* with algae could limit its habitat to areas with warm protected estuaries with abundant algal growth. If so, this may limit its ability to extend its range in the Salish Sea. Studies of coexistence of native and invasive species primarily focus on adult niche partitioning, with exceptional interest in the divergence of adult feeding ecology (Wilson et al., 1999). Investigations into habitat filtration at early developmental stages may provide a new insight into a seldom-considered but potentially widespread mechanism on assessing invasion risks. Contrary to predictions by Hanson et al. (2013a, 2013b), while *H. japonica* may reach patchily large population sizes and densities in warm lagoons and estuaries, *H. vesicula* may be safeguarded from local extirpation by habitat heterogeneity and niche differentiation discussed here should the differences be adequately large. The conspicuous presence of *H. japonica* in False Bay where eelgrass beds were historically abundant further reify inferences of strong associations with macrophytes and macroalgae (Allen, 2014; Gibson, 1993). The current absence of invasive *H. japonica* at False Bay further attests to the resilience conferred to native species by spatiotemporal habitat variation.

H. vesicula *Eggs Released from Cases under Stress in Captivity*

Our data show that *H. vesicula* developed faster than *H. japonica* when reared in captivity under both treatments, exhibiting no compromise in developmental rate in reciprocal lagoon conditions. Nevertheless, we interpret these findings with excess caution and reservations as 1) many egg masses were lost via drifting into the tank outflow and 2) most *H. vesicula* egg masses in the lagoon treatments appeared to have “exploded”, releasing developing eggs instead of hatched veligers into the tank. We omitted these disintegrated egg cases as they are not provisioned for in our scoring schema, leading to the loss of statistical rigor for our lab experiments. The survivorship and hatching rate of the released eggs are not known, though this observation reveals an intriguing line of inquiry.

We speculate that the rupture of egg masses could be an adaptive escape strategy under stressful conditions such that released eggs may drift from adverse conditions. However, it is also possible that the hatching of eggs closer to the exterior of the mass caused a natural deterioration of the clutch, thus freeing the less-developed or unhatched eggs from the center. Experiments designed to test for stressful and conducive environments post-rupture could shed light on whether rupturing egg masses is an adaptive as an escape strategy. Due to the convoluted taxonomic histories of *Haminoea* spp. in the North American Pacific Northwest, it is unclear if *H. vesicula* was displaced from Argyle Lagoon by the invading *H. japonica*, which shows better adaptation to local conditions, or if the former never occurred in the lagoon. Given the high abundance of egg masses we observed in False Bay on myriad substrata, *H. vesicula* may be well-buffered against competitive exclusion by high fecundity and generalist egg-laying strategy, even considering known tradeoffs in increased pathogen risk associated with this strategy (Smoot, 2013).

Cephalaspidean Field Identification Requires Reconsiderations

Our observations of species assemblages differ significantly from historical records, including Gibson (1993), as we noted the absence of both adults and egg masses of *H. japonica* at False Bay. Discrepancies between existing literature (Chaffe & Strathmann, 2005; Russell & Phillips, 2009) and our observations on sites and species occurrence could be due to discordant species concepts amongst authors or displacement of the native species by the invader. This case of mistaken identity prompted us to clarify the taxonomic concepts of these species. Due to the zeal in research on the egg masses of *Haminoea* spp., previous authors may have focused solely on general morphology of egg masses without closely examining differences amongst adults. To verify the match between adults and egg masses, we collected individuals from the field and reared them to egg-laying to match the adults to the egg masses to characterise their morphology. We defend *H. virescens* as distinct from *H. vesicula* due to clear delimitation in adult size, habitat choice and reproductive biology (Fig. 8).

We suspect that previous authors may have misidentified speciesduring field collection and description of reproductive history. Hurst's (1967) description of the morphology of egg masses and developmental schedule of *H. virescens* is distinct from that of *H. vesicula.*

Conclusion

Niche partitioning at early life stages could influence habitat choice in adults, limiting range exploration and expansion in invasive species. Our results reaffirm previous postulations that invader *H. japonica* develops differentially in the presence/absence of macrophytes or macroalgae. *H. vesicula* remains dominant in many habitats from which *H. japonica* is excluded due to low algal cover. The coexistence of this native-invader species pair is not due to paradigmatic competitive coexistence, but rather ontogenetic niche shifts. Furthermore, we furnish here comparative analyses to distinguish the superficially similar egg masses of two native species; furthermore, we recommend prudence and circumspect to field researchers investigating questions in groups with convoluted taxonomic histories.

Acknowledgments

We would miss nothing more than the respective zealous enthusiasm and cautious optimism of course instructors, Richard Emlet and Danny Grünbaum, in disseminating advice, without which much disillusioned digression would have likely taken place. Praise be to course instructor Karen Chan, who despite much-impaired faculty of speech, leveraged alternative communication technology to great effect and comical outcome. We thank Richard Strathmann for his exhaustive wisdom on the history of *Haminoea* spp. on San Juan Island, which reassured the aforementioned instructors of our reticular study concept and design. For *obligatory* camaraderie and commotion, we are indebted to the Larval Biology class of 2023, whose incredible (*i.e., hard to be believed*) penchant for merry-making provided much-needed reprieve from relentless work. To public citizen Jason [last name unknown] who reported our field transplant adrift from its original site, we send our thanks for nebulous directions for recovery and for leaving our egg cases to bake in the sun. YKT and LK thank the benefactor who furnished us with our course tuition and room and board, who wishes to remain incognito, and join them in solemn memory of Mary Rice and Alan Kohn.

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